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Proceedings of the International Symposium on Genetics of Subarctic Fish and Shellfish[1]

May 17–19, 1993
Juneau, Alaska, USA

Eleventh Lowell Wakefield Fisheries Symposium

Compte rendu du symposium international sur la génétique des poissons, des crustacés et des coquillages subarctiques[1]

du 17 au 19 mai 1993
Juneau, Alaska, É.-U.

Onzième symposium sur les pêches Lowell Wakefield

Editors / Directeurs scientifiques

A.J. Gharrett, W.W. Smoker, R.L. Wilmot, J.H. Helle, J.E. Seeb, and L.W. Seeb

Gharrett, A.J., W.W. Smoker, R.L. Wilmot, J.H. Helle, J.E. Seeb, and L.W. Seeb [ed.] 1994. Proceedings of the International Symposium on Genetics of Subarctic Fish and Shellfish [May 17–19, 1993, Juneau, Alaska, USA]. Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 334 pp.

[1]University of Alaska Sea Grant Report No. 95-01.

About the Symposium

The concept for the eleventh Lowell Wakefield symposium to focus on fisheries genetics was submitted to Sea Grant by A.J. Gharrett in late 1990. The symposium was organized and coordinated by Brenda Baxter, Alaska Sea Grant College Program, with the assistance of the program planning committee. Committee members are: Dr. A.J. Gharrett, University of Alaska Fairbanks; Dr. W.W. Smoker, University of Alaska Fairbanks; Dr. Richard L. Wilmot, National Marine Fisheries Service, formerly with the U.S. Fish and Wildlife Service, Dr. J.H. Helle, National Marine Fisheries Service; Dr. James E. Seeb, Alaska Department of Fish and Game; Dr. Lisa W. Seeb, Alaska Department of Fish and Game; and Dr. Carl Burger, U.S. Fish and Wildlife Service.

Sponsors of the symposium and these proceedings are: Alaska Department of Fish and Game; National Marine Fisheries Service; National Park Service; North Pacific Fishery Management Council; U.S. Fish and Wildlife Service; Alaska Sea Grant College Program, University of Alaska Fairbanks. The Alaska Sea Grant College Program is cooperatively supported by the U.S. Department of Commerce, NOAA Office of Sea Grant and Extramural Programs, under grant No. NA90AA-D-SG066, project A/75-01; and by the University of Alaska with state funds.

About the Proceedings

Each paper in this proceedings had at least two peer-reviews. Section editors recommended modifications to authors based on reviews, and approved modified papers as appropriate. Dr. James E. Seeb edited papers on Aquacultural Genetics, Dr. J.H. Helle and Dr. Richard L. Wilmot shared editing of papers on Population Genetics and Evolution, Dr. Lisa W. Seeb edited papers on Evolutionary Processes, Dr. A.J. Gharrett edited papers on Technology, and Dr. W.W. Smoker edited papers on Conservation Genetics. Brenda Baxter coordinated the review process. Thanks to the following reviewers for their time and effort:

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Introduction to Genetics of Subarctic Fish and Shellfish

A.J. Gharrett and W.W. Smoker

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Gharrett, A.J., and W.W. Smoker. 1994. Introduction to genetics of subarctic fish and shellfish. Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 1–3.

Beginning in the 1960s, modern tools of genetic analysis began to be broadly applied in fisheries science. These included techniques ranging from quantitative analysis of pedigreed stocks to population analysis of biochemical genetic traits. Since the end of that decade, research activity in fisheries genetics has quadrupled (Fig. 1). The science of fish and shellfish genetics has had an especially fruitful recent history.

Within the past decade, fisheries genetics has entered the "mainstream" of fisheries resource utilization. Applications of genetic techniques are increasingly common in the early 1990s in both the improvement of aquaculture production and in fishery harvest management, two major resource uses. Aquaculture firms depend on the products of artificial selection for a substantial and increasing part of their seed stock (e.g., Refstie 1990); their trade publications commonly advertise the gender-controlled products of chromosome set manipulation. Managers of some capture–harvest fisheries and of the wild-spawned resources they depend on, particularly Pacific salmon, depend on genetic stock identification techniques both for resource assessment and allocation of harvest to competing fleets (e.g., Pacific Salmon Commission 1992, 1993).

These tools are realizing their potential at a crucial time in world history. In the present decade, demand for fish from the growing world population (Bongaarts 1994a, 1994b) is apparently outstripping the sustainable productivity of capture fisheries, challenging both of these sectors of fisheries genetics (New 1991). Pressures from habitat loss and competing demands for fish protein are increasing the difficulties of natural resource conservation, which will require intensifying use of genetic stock-discriminatory tools. The shortfall in the supply of fish to the world marketplace is estimated at 60 million t yearly by 2025 (New 1991). That shortfall will drive an increase in aquaculture productivity, which is already increasing at 15% per annum (Hershberger 1993). Genetic improvement of growth, fertility, disease resistance, etc., in cultured stocks and conservation of genetic resources will be increasingly important.

This symposium is an assessment of progress and prospects in fisheries genetics, particularly as the science has been applied to subarctic species. The focus on northern species is not overly limiting because much of the pioneering applications of genetics to both fisheries management and aquaculture has taken place in these latitudes, and the movement of genetics out of the laboratory into the workaday world is relatively advanced here. However, it is clear that these trends are global and that neither subarctic fisheries nor subarctic geneticists are isolated. We trust that this formal product of the symposium will serve to accelerate the evolution and use of genetic tools in fisheries science in all communities.

One predominant theme of the symposium was the conservation biologist's and resource manager's concern over maintenance of genetic variability within and among populations. D.L. Hartl's (1994) keynote address contrasted our relatively deep understanding of genetic variability at the species level, such as in the model species of *Drosophila*, with the scant knowledge of differences among species. Although we do not yet have as deep an understanding of genetic variability within species of fish, information is accumulating for a variety of species. Many of the papers presented at this symposium examined genetic variation within and among subarctic species of fish. Such information provides a reference against which future surveys can be compared to detect changes in genetic structure or composition of populations. In many cases the data may also be used for stock identification or separation. The literature is rich in theoretical models that describe the genetic structure and variation of a population using allele frequency information. A review of that literature by Zhivotovsky et al. (1994) outlines some of the caveats for interpreting such data.

As one can see from the descriptive studies included here (Crane et al. 1994; Jorstadt et al. 1994; King et al. 1994; Kondzela et al. 1994; Phelps et al. 1994; Shaklee and Varnavskaya 1994; Varnavskaya et al. 1994a, 1994b; Wilmot et al. 1994; Winans et al. 1994; Wood et al. 1994), the number of allozyme loci and the alleles detected at those loci have increased dramatically in the last three decades. Four papers combine to present allozyme descriptions of chum salmon (*Oncorhynchus keta*) throughout much of the natural range (Kondzela et al. 1994; Phelps et al. 1994; Wilmot et al. 1994; Winans et al. 1994). Because it is unusual to have such a complete picture of a single species in one volume, the *Canadian Journal of Fisheries and Aquatic Sciences* has allowed us to include data tables that are somewhat more extensive than usually accompany a paper.

Other descriptive studies report on the development and use of DNA markers. Analysis of mitochondrial DNA (mtDNA) from both restriction fragment patterns (Adams et al. 1994; Bernatchez and Dodson 1994; Danzmann et al. 1994; Gold et al. 1994) and direct sequencing (Nielsen et al. 1994) has been augmented by the use of nuclear DNA markers. The approaches reported for accessing the nuclear variability include DNA fingerprinting using probes derived for use in other species (Spruell et al. 1994; Leung et al. 1994), isolation of species-specific probes (Withler et al. 1994), analysis of the species specificity of highly repeated satellite DNA sequences (Hartley and Davidson 1994), and sequence analysis of ribosomal DNA spacers (Phillips et al.

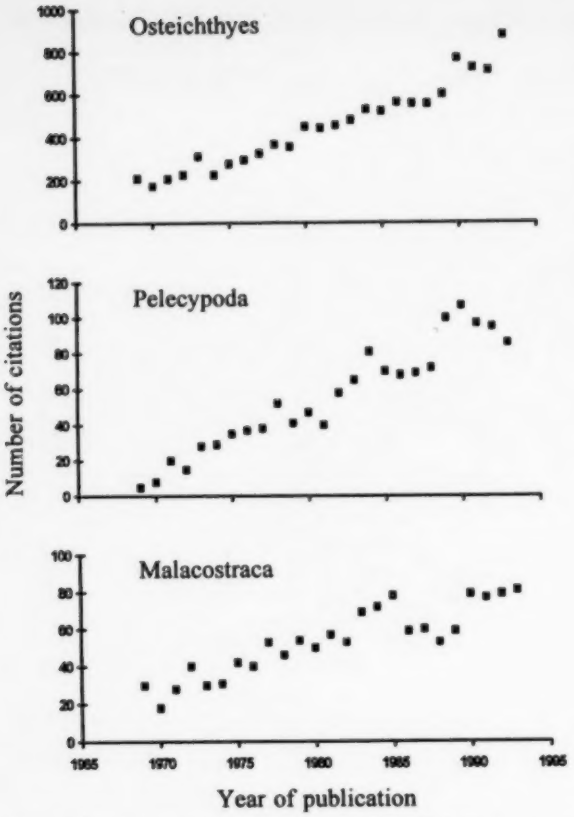

FIG. 1. Number of citations listed in Biosis™ of publications on cytogenetics and population genetics of taxa including fish, molluscs, and crustaceans each year from 1969 to 1993.

1994). These new methods promise to unlock an extraordinary amount of genetic variability and permit geneticists to ask detailed questions about the hierarchical nature of population structure in a species, including questions about the definition of the term "stock" (STOCS 1981).

Another focus of the research has been polygenic or quantitative genetic variation rather than variability at single loci (Smoker et al. 1994). Whereas biochemical genetic variation at individual loci is often presumed to be neutral or nearly neutral (e.g., Nei 1983), quantitative genetic variation of traits (such as life-history traits) can be strongly influenced by natural selection. Although insight into quantitative genetic variation may be much more difficult to obtain than allele frequency data, such variation lies at the heart of genetic divergence among populations, which can exist even when substantial gene flow occurs among populations.

Human activities broadly threaten biodiversity, including that of aquatic systems (e.g., Culotta 1994). Although losses and degradation of habitat are the major part of the threat to biodiversity, there are other contributing factors. There has been notable concern among fisheries scientists about the effects on biodiversity of fish culture in hatcheries. As shown by Nielsen et al. (1994), domestication of a cultured species may lead to loss of genetic variability, either by purposeful or inadvertent selection. Interactions between cultured and self-sustaining or "wild" populations may jeopardize the wild populations. Waples and Do (1994) model some of these interactions to demonstrate the magnitude of the effect of interaction between cultured and wild stocks.

A method for mitigating the potential influence of cultured fish on wild stocks is to culture sterile fish. Sterility can be induced genetically by producing triploids or interspecies hybrids. In addition to sterility, interspecies hybrids may offer advantageous traits of both parental species. Several papers examined the effects of triploidization, hybridization, and performance of the hybrid and triploid products (Galbreath and Thorgaard 1994; Habicht et al. 1994; Joyce et al. 1994; Miller et al. 1994; Teplitz et al. 1994).

Concern for the conservation of the genetic variation of fish and shellfish stocks begins with understanding the nature of population structure and variation among populations (Gresswell et al. 1994). Certainly, there are sufficient examples of populations that are near extinction to stimulate concern and activity that will help us avoid endangering other populations in the future. Obviously, these concerns and activities cannot compensate for habitat loss; however, detailed knowledge of intra- and inter-populational structure should play an important role in decisions affecting the resource. It is inevitable that the number of threatened stocks will grow. Stemming declines in some of these populations, rehabilitating others, and conserving the genetic heritage of the species will require a monumental effort. Now, while we have the opportunity to study successful self-sustaining populations, it is imperative that we learn as much as we can about the genetic structures that contribute to the success of a population.

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Keynote Address
Macro and Micro in Molecular Evolution

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It is an honor to be the keynote speaker at the Symposium on Genetics of Subarctic Fish and Shellfish. Geneticists working with fish and shellfish have for many years maintained a consistently high level of sophistication and expertise in their research, and they have been aggressive in the best sense of the word in capitalizing on discoveries in basic genetics. This is a record of achievement of which everyone in the profession can take great pride. It is indicative of the breadth of interest of this audience to have invited a keynote speaker who has worked principally with molecular evolution in *Drosophila*. It therefore occurred to me that I might contribute most effectively to this meeting by addressing the broad theme of "macro and micro in molecular evolution." I will discuss several broad issues in evolutionary biology and emphasize the manner in which molecular evolutionary genetics can open up new approaches to these problems.

One general issue is the well known dichotomy between microevolution and macroevolution. Table 1 shows a selected few of the concepts from each of the fields; microevolution above and macroevolution below. The lists are by no means exhaustive, and the terms are ordered alphabetically to avoid any implication of a hierarchy of importance. Microevolution deals mainly with the nature and causes of genetic variation within and between subpopulations of the same species. The concepts fundamental to microevolution include mutation, migration, selection, random genetic drift, and other classical processes forming the core of population genetics (Hartl and Clark 1989). The microevolution list also includes topics of much recent interest, such as coalescence theory, the search for loci influencing quantitative traits, and the nature and amount of selection resulting in biased codon usage. Major concepts dealing with macroevolutionary change are listed on the bottom of the ledger; these include genetic differences between species, mechanisms of speciation, adaptation, extinction, developmental constraints, stasis, species selection, and so forth.

Although a number of the processes in Table 1 are understood at an impressive level of detail, there is virtually no insight flowing between the microevolutionary and macroevolutionary parts of the table. Difficult as it is to draw specific connections between microevolution and macroevolution, one of the important things for evolutionary biology in the next century is to make closer connections between evolutionary processes within populations and those that determine the broader evolutionary patterns.

The connections between microevolution and macroevolution are important because one of the principal contentions of Darwinism is still at issue. I do not mean the efficacy of natural selection: nobody (not even creationists) disputes the ability of natural selection to change the frequencies of genotypes in populations and, therefore, the genetic characteristics of populations. The major issue still unresolved is whether natural selection alone can explain the major features of life on earth, including the organizational patterns of living beings and the changeovers from one preponderant set of life forms to another. It is now clear that mass extinctions account for some of the major changes in the history of life, and the persistence of species through such upheavals may often be governed by survival of the luckiest rather than survival of the fittest. Mass extinctions are very infrequent, however, and account for only a small proportion of all extinctions.

A second issue I want to address is that of molecular evolution. One could make a table resembling Table 1 including an extensive set of processes related to molecular evolution at the micro level: these would deal with the molecular evolution of structural genes, promoters, and enhancers; the origin and fate of gene duplications; the molecular evolution of transposable elements; the use of DNA and protein sequences to infer molecular phylogenies; and so forth. In other words, the list would include evolutionary aspects of most of the processes that fill the literature of genetics and molecular biology.

There would also be a ledger related to molecular evolution at the macro level. This would include such topics as life in the RNA world and its transition to the DNA world, the origin of proteins with complex functions by combinatorial means from sequences coding for distinct folding domains, the emergence of multicellularity and the differentiation of distinct cell types, the molecular evolution of the handful of distinct metazoan body plans that first appeared in abundance in the Cambrian, and the molecular mechanisms of developmental constraint. Once again, the limited insight flowing between the understanding of molecular evolution at the micro level and that at the macro level is a disappointment.

There is some hope that the gap in molecular evolution between the micro and the macro level can be narrowed, in part by detailed study of the genomes of certain model organisms (Watson 1990; Collins and Galas 1993). For some years, my laboratory has been involved in the genome project of the fruit fly, *Drosophila melanogaster*, and the project has finally reached a point at which its materials are important resources for evolutionary research. Furthermore, DNA sequencing of the entire *Drosophila* genome is already underway. Figure 1 shows a clock analogy for the dates at which genomic sequencing would be completed if sequencing of various model organisms had begun January 1, 1994, and progressed at the rate of 6 base pairs per second. Phage lambda would be finished at 2:20 a.m., *Escherichia coli* on January 10, yeast on January 29, and the euchromatic genome of *Drosophila* and the genomes of *Caenorhabditis* and *Arabidopsis* would each be finished on or about July 12 in this analogy. (Including the heterochromatin of *Drosophila* would

extend the project to November 14.) Because the true rate of progress in most sequencing laboratories is presently more like 6 base pairs per minute, the projects are all going to take a little longer than shown in Fig. 1.

The *Drosophila* genome project has made extensive use of clones containing large inserts of exogenous DNA, such as yeast artificial chromosomes (YACs), and bacteriophage P1 (Hartl 1992; Hartl et al. 1994). The method has made extensive use of in situ hybridization of the cloned DNA to the giant polytene chromosomes in the nuclei of third instar larvae. These chromosomes are an extraordinary cytogenetic feature of *Drosophila* and other dipterans. They are giant chromosomes by any standards. In some dipterans, although not in *Drosophila*, the polytene chromosomes are large enough to see with the naked eye. This includes some of the Alaskan blackflies in the family Simuliidae. The polytene chromosomes are formed from somatic pairing of the extended homologous chromosomes and about a tenfold lateral replication, yielding about 1000 aligned copies of each DNA molecule. However, part of the genome is underreplicated in the salivary gland nuclei and remains clumped together in a nondescript mass called the chromocenter. The chromocenter includes the pericentromeric heterochromatin and the Y chromosome. Much of the DNA in this heterochromatic part of the genome has a very simple structure of highly repeated satellite DNA sequences, such as the motifs AATAT or AAGAG, repeated in tandem hundreds of thousands or millions times (Lohe et al. 1993). However, the vast majority of the genes reside in the euchromatin, which forms the banded part of the polytene chromosomes.

There are about 5000 bands in the chromosomes of *Drosophila* and also about 5000 complementation groups ("genes") in the *Drosophila* genome. Through the years there has been a long discussion of the relation between genes and bands, and the relation is still unclear (reviewed in Baker et al. 1991). In fact, the considerations have become more complex because the estimated 5000 complementation groups yield about 15 000 transcripts. Therefore, it is not only unclear how genes correspond to bands but also unclear how transcription units correspond to genes as well as to bands.

The polytene chromosomes have been a principal tool for genetic analysis in *Drosophila* since they were discovered in the 1930s (Painter 1933). Every evolutionary geneticist knows the seminal work of T. Dobzhansky on the polymorphic inversions in *D. pseudoobscura* and related species (summarized in Anderson et al. 1991). The reason the polytene chromosomes are so valuable is that the banding patterns are linear. Indeed, as stated by Stone (1962): "The polytene chromosomes are the largest and most complex ordered living system with a determined and consistent inherited pattern that is changed in geometrically simple and limited ways through two-break rearrangements. No other cellular organelle in natural populations of living organisms has an equivalent ordered complexity in which changes can so easily be determined. Therefore chromosome evolution in the genus *Drosophila* and in other Diptera with polytene chromosomes is most readily and accurately measured."

It is worth pointing out here that the features of linearity in structure and (usually) simple mechanisms of evolutionary change have also made DNA such an important molecule for molecular evolutionary studies. Chloroplast DNA in plants, mitochondrial DNA in plants and animals, and chromosomal DNA in all organisms provide the primary data for contemporary molecular phylogenies, just as the polytene chromosomes did for *Drosophila* chromosomal phylogenies a few decades earlier.

TABLE 1.

Microevolution
Coalescence, codon bias, genetic covariance
Genetic variance, heritability, mating systems
Meiotic drive, migration, mutation
Random genetic drift, recombination
Selection, quantitative trait loci, variation within populations
Macroevolution
Adaptation, adaptive radiation, constraints
Convergence, development, ecological opportunity
Extinction, mass extinction, morphological change
Speciation, species selection, spindle diagrams
Stasis, variation between species

On 11 October 1991, the journal *Science* published a summary of the status of studies of the *Drosophila* genome project, as well as a fold-out poster summarizing the information (Merriam et al. 1991). The poster featured the cytological positions of approximately 1200 yeast artificial chromosome clones having an average insert size of about 200 kilobase (kb) pairs of DNAs. Based on best judgments of whether two nearby YACs contain DNA from overlapping sets of polytene bands, the YAC clones have been grouped into 149 cytological contigs of average size 638 kb; 26 of the cytological contigs exceed 1 megabase (Mb), and the maximum size is 4.2 Mb. The cytological contigs are separated by 154 gaps, among which the median size is 50 kb. Altogether, approximately 90% of the euchromatic genome is included in the mapped YACs (Hartl 1992; Hartl et al. 1992; Hartl and Lozovskaya 1992). The Science poster also included the locations of numerous cosmid contigs, especially along the X chromosome (Sidén-Kiamos et al. 1990; Kafatos et al. 1991), and chromosome walks that had been carried out with smaller cloning vectors. The extensive data cited in this review (Merriam et al. 1991) results from the fact that *Drosophila* has been of substantial interest to geneticists for more than 80 yr. During this period geneticists have generated a source of research materials that is unparalleled in its ability to manipulate organisms for genetic analysis. The tabulation in Merriam et al. (1991) includes a total of 3758 known genes, 17 970 chromosomal breakpoints, and numerous insertion points of transposable elements.

The YAC cloning system has been very useful for working with extremely large genomes, such as the human genome (Cohen et al. 1993). For purposes of genome analysis in *Drosophila*, there is a trade-off between size of clone and technical ease, which tips toward the side of smaller clones that are easier to work with. In the *Drosophila* genome project, we have switched to an alternative cloning vector based on bacteriophage P1 (Pierce et al. 1992; Smoller et al. 1991). P1 clones contain DNA inserts that average approximately 80 kb, but the host organism is *E. coli* and the clones yield large quantities of high purity DNA suitable for subcloning, DNA sequencing, and other purposes.

We have generated a second-level framework map of the *Drosophila* genome based on 2461 bacteriophage P1 clones ordered by in situ hybridization (Hartl et al. 1994). The

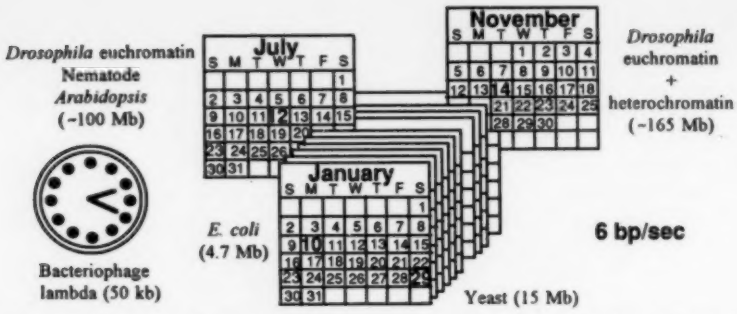

FIG. 1. Completion dates for sequencing of various model organisms given a start date of January 1, 1994, and a rate of 6 base pairs per second. (The actual rate is presently approaching 6 base pairs per minute.)

P1 map includes an estimated 85% of the sites in the euchromatic genome with an average depth of coverage of 1.8. The mapped P1 clones, as well as 6755 additional clones, are currently being screened with sequence-tagged site (STS) markers in order to be arrayed in molecular contigs. The localizations of P1 clones and other information, as well as sources from which P1 clones may be obtained, are available to the scientific community by anonymous FTP (file transfer protocol) from ftp.bio.indiana.edu in the directory flybase or by electronic mail requests to the address flybase@morgan.harvard.edu. There are no restrictions on the research uses of the material.

The *Drosophila* physical map is a wonderful resource for research in evolutionary biology (Hartl et al. 1994). For example, the YAC and P1 clones already isolated include the breakpoints that distinguish the chromosomes of *D. melanogaster* from those of closely related species, such as *D. simulans*. Furthermore, the clones include the breakpoints of inversions that are polymorphic worldwide in populations of *D. melanogaster*. Thus, these materials afford molecular access to the inversion breakpoints and insight into the processes by which inversions in natural populations are formed.

Although the chromosome banding patterns of more distantly related species, such as *D. pseudoobscura* are too different to assign homologies by their morphology, more than 80% of the P1 clones from *D. melanogaster* yield strong, single hybridization patterns with the salivary gland chromosomes of *D. pseudoobscura* (C. Segarra and E.R. Lozovskaya, unpublished observations). Hence, the framework map of *D. melanogaster* provides the material for a detailed alignment of the *D. melanogaster* and *D. pseudoobscura* euchromatic genomes. These species have undergone an estimated 30 to 40 million yr of evolutionary divergence (Beverley and Wilson 1984), and I am unaware of any cytogenetic comparative mapping that is possible at such a high resolution in any other group of organisms.

Drosophila pseudoobscura, like *D. melanogaster*, is in the subgenus *Sophophora*. More distantly related species are found in the subgenus *Drosophila*, which diverged from *Sophophora* an estimated 60 million years ago (Beverley and Wilson 1984). To investigate evolution at this level, we have chosen *D. virilis* (one of the classical species subgroups studied by Patterson and Stone 1952; see also Patterson et al. 1940). The euchromatic genome of *D. virilis* is about 40% larger than that of *D. melanogaster*. Why is the genome larger in one species than in the other? Where is the extra DNA located? Is it between genes? Is it in the introns? Are the genes, on the whole, longer? Is there any functional reason for the larger genome?

To begin to address these issues, we first attempted in situ hybridization with P1 clones from *D. melanogaster*. This approach proved unsuccessful in our hands, we suppose because the DNA sequences between the species are too divergent. However, the methods of genome mapping used for *D. melanogaster* are equally applicable to *D. virilis*, and so we made a P1 library containing inserts of *D. virilis* and began constructing a framework physical map (Lozovskaya et al. 1993). The framework maps of the two species can be correlated because, although in situ hybridization with the polytene chromosomes does not work, colony hybridization with P1 clones does. Hence, we have also used probes from *D. melanogaster* generated by the polymerase chain reaction in order to screen the *D. virilis* P1 library (Lozovskaya et al. 1993). Among the *D. virilis* genes that we have isolated are homologs of the *D. melanogaster* genes *hunchback*, *yellow*, *mastermind*, and many others. One result is already clear: the genome of *D. virilis*, relative to that of *D. melanogaster*, is highly rearranged, and it will be of great interest to determine, if possible, the distribution of segment lengths that have remained intact in the genealogy of these species. Is there any functional significance to the reorganization of the genome map? Are certain linkages conserved in spite of the scrambling? Where are these in the genome and what genes do they contain?

A second use of the *D. virilis* homologs of *D. melanogaster* genes is that they can be used to begin to address the question of how rapidly evolution occurs at the level of gene function. To what extent do genes that have been in separate lineages for 60 million yr diverge in function? On the whole, do homologs of genes from *D. virilis* complement mutations in *D. melanogaster* ? If they do, what is the quantitative level of complementation? Are certain classes of genes more conserved functionally than others? In spite of there being some spectacular examples of complementation of genes in organisms even more distantly related than *D. melanogaster* and *D. virilis* , these examples involve functions that are highly conserved in evolution, and I have no doubt that many genes for fundamental process in cell biology and development will be found to be functionally interchangeable even in very disparate organisms. However, the sampling of genes for cross-species complementation

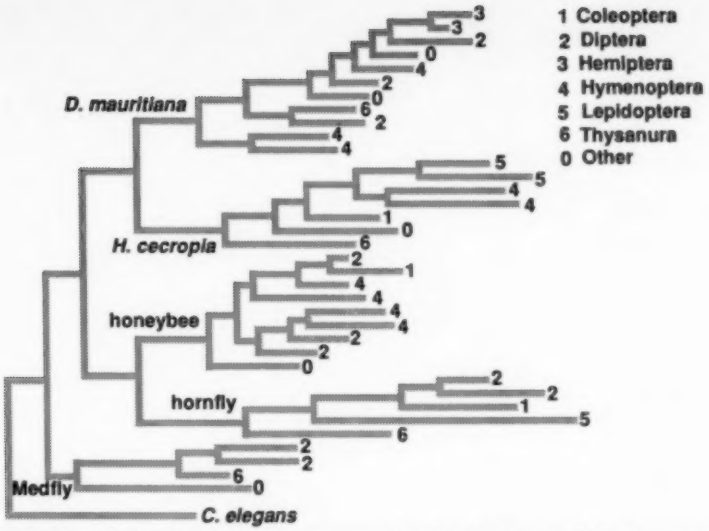

FIG. 2. Molecular relationships of DNA sequences related to the transposable element *mariner* in various orders of insects. Data from Robertson (1993).

is highly biased and extremely small; there is also a bias in the reporting because lack of complementation is not a publishable result whereas complementation is. Therefore, I believe that reliable inferences about gene function will require a much wider sample of genes. There must certainly be some divergence of function, else why would species be different?

To overcome the barriers of reproductive isolation in order to transfer genes between species, *Drosophila* offers a number of systems based on transposable elements, including the P element (Rubin and Spradling 1982) and the *hobo* element (Smith et al. 1993). These systems are excellent for introducing genes into the germline of *D. melanogaster*, but they may be limited in their applicability to other species because they are highly restricted in their phylogenetic distribution. One element with a potentially wider host range is the *mariner* transposable element, originally discovered in *D. mauritiana* and other species of the *D. melanogaster* subgroup (Hartl 1989; Bryan et al. 1990; Capy et al. 1992a, 1992b).

The *mariner* element is small (1286 base pairs), contains a single, long open reading frame, and is active in somatic cells as well as germ line cells (Hartl 1989). The *mariner* element is present not only in drosophilids but also in the very distantly related dipteran *Zaprionus* (Maruyama and Hartl 1991), in the wild silkworm *Hyalophora cecropia* (Lidholm et al. 1991), in an astonishingly wide variety of insects and other invertebrates (Robertson 1993; Robertson and MacLeod 1993), as well as in humans and some other vertebrates (H.M. Robertson and A.R. Lohe, unpublished observations). Figure 2 summarizes some of the data of Robertson (1993) indicating the presence of *mariner* in most major orders of insects. The widespread nature of this distribution, as well as the strong support for horizontal transmission (Maruyama and Hartl 1991; Lawrence and Hartl 1992; Robertson 1993; Capy et al. 1994), strongly suggests that *mariner* requires very little of its host (except perhaps functions that are strongly conserved in evolution), and so might be developed into a generalized transformation vector, at least for invertebrates.

The development of the *mariner* system is still in progress, but what we know already is that *mariner* elements from *D. mauritiana* readily transpose into the germ line of *D. melanogaster* (a species in which the *mauritiana* type of element does not occur) (Garza et al. 1991) and, moreover, that the *mariner* element can mobilize exogenous DNA (Lidholm et al. 1993). Work in progress includes optimizing the conditions for transformation and the size and position of the exogenous DNA. If the initial promise of the *mariner* system is realized, then it will open up functional evolutionary analysis not only between *D. melanogaster* and *D. virilis*, but also between many other groups of insects and, more generally, the Invertebrata.

What I tried to address in this presentation are broad questions of evolutionary biology that can be approached using some of the technology of genome mapping and the molecular genetics of transposable elements. My hope is that the future of evolutionary biology, extending well into the next century, will be dominated not by studies of DNA sequence but by studies of DNA function in order to understand how much real functional difference there is in the genes of distinct organisms.

Acknowledgments

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Genetic Aanalysis of Size in an Anadromous Population of Pink Salmon

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Variation of size, particularly among males, has a significant genetic basis in pink salmon *(Oncorhynchus gorbuscha)* in Auke Creek, southeast Alaska. Heritability (h^2), based on variance components of 118 full sib – 59 paternal half sib families of mature fish tagged as fry with coded micro wires, are higher in males (h^2 length: 0.8 ± 0.3 (mean ± SE); h^2 weight: 0.6 ± 0.2, based on sire effect) than in females (h^2 length: 0.3 ± 0.2; h^2 weight: 0.4 ± 0.2). Realized heritability probably would be smaller because of environment variability between brood years in factors affecting size and growth. Estimates based on regression of offspring means on fathers' values are smaller (h^2 length: 0.4 ± 0.1 in males; 0.2 ± 0.1 in females; h^2 weight: 0.0 in males and 0.1 ± 0.1 in females). Estimates of genetic, environmental, and phenotypic correlations of length and weight are all >0.7 (SEs <0.1). Estimates of genetic correlation between length and day of migration from the sea are near 0.4 ± 0.2; estimates of environmental and phenotypic correlations between these traits are smaller (<0.2, SEs <0.1).

Chez le saumon rose (*Oncorhychus gorbuscha*) du ruisseau Auke, dans le sud-est de l'Alaska, la variation de la taille repose sur une base génétique importante, surtout che les mâles. L'héritabilité (h^2), fondée sur des éléments de variance mesurés chez 118 familles pleinement apparentées – 59 familles à demi-apparentées du côté paternel de poissons matures marqués au stade d'alevin à l'aide demicromarques magnétisées codées, est plus élevée chez les mâles (longueur h^2 : 0,8 ± 0,3 écart-type (é-t); poids h^2 : 0,6 ± 0,2, fondé sur l'effet de géniteur) que chez les femelles (longueur h^2: 0,3 ± 0,2; poids h^2 : 0,4 ± 0,2). L'héritabilité réalisée serait probablement plus faible à cause de la variabilité des facteurs environnementaux touchant la taille et la croissance entre les années de génération. Les valeurs estimées fondées sur la régression de la moyenne de la progéniture sur les valeurs propres aux pères sont plus faibles (longueur h^2 : 0,4 ± 0,1 chez les mâles et 0,2 ± 0,1 chez les femelles; poids h^2 : 0,0 chez les mâles et 0,1 ± 0,1 chez les femelles). Les valeurs estimées des corrélations génétiques, environnementales et phénotypiques sur la longueur et le poids sont toutes inférieures à 0,7 (é.t. <0,1). Les valeurs estimées des corrélations génétiques entre la longueur et le jour de montaison se rapprochent de 0,4 ± 0,2, alors que les valeurs estimées des corrélations environnementales et phénotypiques entre ces traits sont inférieures (<0,2, é.t. <0,1).

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Genetic analysis of size in fish in their natural environment is important for at least two practical reasons. The genetic consequences of selective harvest are a concern of fishery scientists and managers whose aim is to protect the fitness and productivity of stocks (e.g., Sutherland 1990; Policansky 1993). Genetic improvement of stocks in ocean-ranching or extensive culture may be possible through broodstock management (Gjedrem 1986). Estimates of pertinent genetic parameters in either harvested wild stocks or ocean-ranched stocks have been few. In salmonids, genetic parameters related to size have been estimated for cultured populations of Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), coho salmon (*O. kisutch*), and others (e.g., Gall 1975; Gjerde and Gjedrem 1984; Gjerde and Shaeffer 1989; Hershberger et al. 1990). In wild-spawned or ocean-ranched stocks, several researchers have studied traits of embryonic and larval stages (McIntyre and Blanc 1972; McIntyre and Amend 1978; Smoker 1986; Beacham and Murray 1988), and some have studied captive fish sampled from wild populations (Beacham and Murray 1988; McKay et al. 1986). Few have attempted to study mature fish growing in their native environment (McIntyre et al. 1988; Hankin et al. 1993; Smoker et al. In press). Direct studies of the inheritance of harvestable size of salmon in their natural environment have not been attempted.

We studied the inheritance of body size at maturity in a population of anadromous pink salmon, using a standard half-sib – full-sib breeding design (Becker 1984) to estimate heritabilities and genetic correlations of length and

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weight of adult salmon and to estimate genetic correlation between size and timing of return (Smoker et al. In press). We mated each of 60 male pink salmon (*Oncorhynchus gorbuscha*) to two females (120 total) and released their offspring, tagged with family-specific coded micro wires. The fry were released without rearing and commingled with wild-spawned fry that were emigrating to the sea from the same population. We intercepted surviving adults as they made their anadromous migration to fresh water and measured them.

Study Site

Auke Creek (Juneau, Alaska) and its pink salmon populations have been described by Taylor (1980) and Lane et al. (1990); it is short (350 m), steep (falling 20 m), and lake fed. Since 1971 records have shown that 2000 – 26 000 adults enter Auke Creek between late July and late September and spawn within a few days. Two groups of adults enter each year, separated by a hiatus near 1 September, in both odd and even years. Fry leave in a roughly unimodal emigration between late March and early May. (Data are on file at U.S. NMFS Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801, USA.) The ancestry of Auke Creek pink salmon includes some fish released as unfed fry from artificial incubators for three generations in the late 1970s, but it is in other respects a natural population. A research weir and a research hatchery permit control, culture, and observation of salmon. The weir and trap for anadromous adults is immediately upstream from the intertidal mouth of Auke Creek.

Methods

We made 60 half-sibling and 120 full-sibling families by hierarchical mating of 60 males, each with two different females (Smoker et al. In press). The parents were chosen by random sample from the 1983 run in Auke Creek. Half of the parents were sampled and spawned together near the peak of migration on 17 August. Half of the parents were spawned near the later peak on 20 September. On each occasion we sequestered returning fish for 3 d, sequentially removed the known number (above 250) of mature fish from holding pens, and used pseudo-random numbers to choose the parents (our algorithm for generating pseudo-random numbers is based on a combined congruential–shift register generator suggested by Ralston and Reilly (1983), and is available from A.J. Gharrett). Incubation of each full-sibling family of embryos took place in three replicate containers assigned at random to positions in an array of F.A.L. Heath™ incubators.

At completion of development (ventral sutures closed, before exogenous feeding) technicians tagged all or a sample of fry from each family with coded micro wires, one code per family (Thrower and Smoker 1984). The number tagged ranged from 56 to 1138 per family, 87 895 in all. All tagged fish were marked by excision of the adipose fin. The average weight of the unfed fry was 0.25 g. We released the August-spawned families on 28 March and the September-spawned families on 3 April 1984, during the emigration of wild pink salmon fry from Auke Creek.

During 27 July – 20 September 1985 operators of the weir examined each of 26 000 returning pink salmon; 1890 were marked by missing adipose fins. Of those marked adult pink salmon, 1307 retained tags. Technicians of the Alaska Department of Fish and Game Tag Laboratory recovered and decoded the tags using their standard quality control procedure, which requires agreement between two independent decoders. The 1307 salmon represented 118 full-sib – 59 half-sib families.

We recorded lengths of all 1307 tagged fish and weights of 1144 fish. Because secondary sexual characters distort the shape of the head of mature pink salmon, we measured length from the middle of the eye to the fork of the tail in millimeters. We measured weight to the nearest 100 g.

For both males and females we used a nested statistical model to analyze variation in length or weight:

$$\text{Size (length or weight)} = \mu + P_i + S_{j(i)} + D_{k(ji)} + e_{ijkl}$$

where μ is the population mean size, P_i is the fixed effect of the ith date of spawning (by the parents of the observed fish), $S_{j(i)}$ is the random effect of the jth sire within the ith date of spawning, $D_{k(ji)}$ is the random effect of the kth dam mated to the jth sire, and e_{ijkl} is residual error associated with the lth offspring in each family.

We also analyzed independent subsets of the data, early-spawned and late-spawned fish, separately; those models were without the fixed effect of spawning date, P. We used the GLM procedure in SAS Institute Inc. (1989) to compute all analysis of variance.

We tested significance of effects by approximate F-tests (Searle 1971, Ch. 6) and estimated components of variance of, heritabilities of, and the genetic, environmental, and phenotypic correlations between length, weight, day of return, and standard errors of those parameters, from the intraclass correlations according to formulas given in Becker (1984).

We estimated heritability as the ratio of additive genetic variance to total phenotypic variance:

$$(1) \qquad h^2 = V_A/V_P$$

where, absent non additive genetic effects, $V_A = 4\,\sigma_s^2$ or four times the sire component of variance and $V_P = \sigma_s^2 + \sigma_d^2 + \sigma_e^2$ or the sum of the sire, dam, and error components of variance. Formulae for standard errors are given in Becker (1984, p. 53–63).

We also estimated heritabilities from the coefficients of regressions of offspring means on sire, dam, or midparent values (Becker 1984 p. 101–104):

$$(2) \qquad h^2 = 2b_{op}$$

where b_{op} is the regression coefficient of family mean size regressed on sizes of sires or of dams.

The first estimate (Equation 1) involves correlations between half siblings and assumes that all of them experienced the same environmental conditions on average. The second estimate (Equation 2) requires the same assumption about parents and offspring.

Formulae for estimating genetic (r_G), environmental (r_E) and phenotypic (r_P) correlations and their standard errors are also from Becker (1984p. 111ff).

Results

The date of spawning (17 August or 20 September) affected the size of returning adults (Table 1). Late-spawned fish were longer and heavier than early spawners (male length: $F_{1,57} = 8.59$, $P = 0.0049$; male weight: $F_{1,57} = 7.47$, $P = 0.0083$; female length: $F_{1,57} = 20.39$, $P = 0.0001$;

TABLE 1. Mean length and weight (standard deviations in parentheses) of early-spawned and late-spawned pink salmon. Tagged adults returning in 1985. Numbers of observations (n).

	Early-spawned		Late-spawned	
	Length (mm)	Weight (kg)	Length (mm)	Weight (kg)
Males	452.32	1.26	469.51	1.42
	(29.85)	(0.30)	(29.02)	(0.33)
n	293	293	429	348
Females	467.94	1.38	483.01	1.53
	(25.33)	(0.24)	(21.97)	(0.23)
n	262	261	322	242

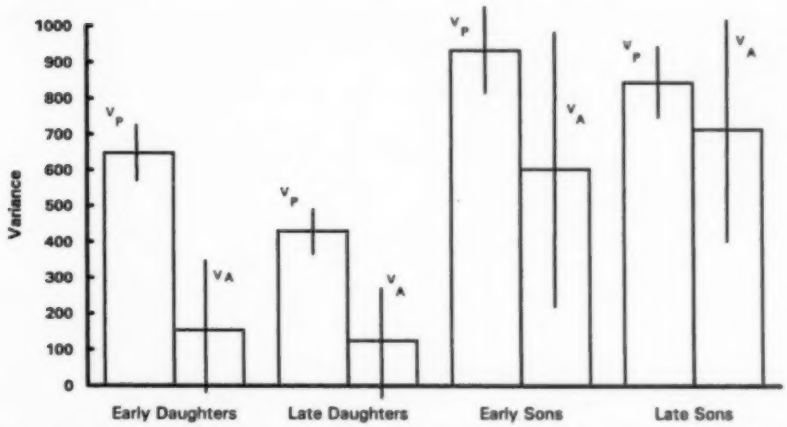

FIG. 1. Phenotypic (V_P) and additive genetic (V_A) variation of adult length in early spawned (17 August 1983) and late-spawned (20 September) pink salmon from Auke Creek. V_P was estimated as the sum of sire, dam, and error components of variance. V_A was estimated as four times the sire component of variance. Units are square millimetres. Standard errors indicated by vertical bars.

female weight: $F_{1,57} = 15.97$, $P = 0.0002$). (Variance analyses are given in the Appendix.)

A genetic basis for variation of male offspring size was shown by significant variation due to sires; however, variation among female offspring is less significant (males: length, $F_{57,48} = 2.25$, $P = 0.0023$; weight, $F_{57,48} = 1.99$, $P = 0.0077$; females: length, $F_{57,50} = 1.35$, $P = 0.1393$; weight, $F_{57,50} = 1.62$, $P = 0.0417$). Estimated phenotypic and additive genetic variation of length among individuals was greater among males than among females in both early- and late-spawned fish, (from separate analysis of early- and late-spawned groups; Fig. 1). The gender differences were repeated in independent groups. Among females phenotypic variation was less in late-spawned fish than in early-spawned fish, but timing-related differences were not evident among males (Fig. 1).

Intraclass correlation estimates of heritability of length or weight were above 0.5 in males but less than 0.4 in females (Table 2). Estimates of heritability of length from regression of offspring on parents were less, 0.4 in males and 0.15 in females; similar estimates of heritability of weight were even smaller (Table 2).

Genetic, environmental, and phenotypic correlations of length and weight were all high, approaching unity, in both male and female offspring (Table 3). Genetic correlation of length and day of return was near 0.4 in both males and females, but environmental and phenotypic correlations were low (Table 3).

Discussion

Our estimates of heritability of size for males in an anadromous population of pink salmon, when calculated from intraclass correlations, are higher than published estimates for captive salmonids, which tend to be between 0.2 and 0.4 (e.g., Gall 1975; Gjerde and Gjedrem 1984; Gjerde and Shaeffer 1989; McKay et al. 1986). Environmental contributions to variability during 16 mo at liberty in the ocean would be expected to be different than environmental contributions in captive culture. If ocean variability were greater it would tend to deflate heritability in anadromous salmon because of the increase in total phenotypic variability. It seems unlikely that environmental variability would be reduced in families at liberty in the ocean but would be increased compared with the relatively homogenous controlled conditions of captive culture. It may be that genetic variability of size is greater in anadromous Auke Creek pink salmon than in other salmonids. Beacham and Murray (1988) also observed high heritability of size in male pink salmon whose parents were sampled from an anadromous population, although they observed growth and size in captive individuals. Substantial genetic variability of size in males may be characteristic of pink salmon populations.

Our estimates of heritability of size from regression of offspring mean values on parent values are less than those from intraclass correlations, even when all of our data including

TABLE 2. Heritability (h^2) of length, weight, and day of return to fresh water in male and female Auke Creek pink salmon based on sire (s), dam (d), or sire and dam (sd) effects or based on regressions of offspring means on sires, dams, or midparent. Standard errors are given in parentheses. Broemeling's (1969) 95% simultaneous lower confidence limit on the estimate of heritability based on the sire effect (95% LCL). Full Model: all data including families from early and late spawning dates analyzed together. Early: families from 17 August spawning date. Late: families from 20 September spawning date. See Smoker et al. In press. This analysis includes only observations matching all three traits (length, weight, and day of return), the same data used to estimate correlations presented in Table 3.

	Males			Females		
	Length	Weight	Day	Length	Weight	Day
Full model including spawning date as the main effect (118 full-sib families)						
Sib analyses						
h_s^2	0.76 (0.27)	0.61 (0.25)	0.19 (0.17)	0.26 (0.22)	0.36 (0.20)	0.37 (0.22)
95% LCL h_s^2	0.77	0.61	0.13	0.19	0.16	0.25
h_d^2	0.53 (0.22)	0.50 (0.22)	0.28 (0.20)	0.38 (0.26)	0.13 (0.22)	0.30 (0.24)
h_{sd}^2	0.55 (0.15)	0.50 (0.22)	0.23 (0.11)	0.32 (0.14)	0.24 (0.13)	0.34 (0.14)
Offspring–parent regression						
Offspring–sires	0.30 (0.12)	0.00 (0.00)		0.16 (0.06)	0.00 (0.08)	
Offspring–dam	0.46 (0.16)	0.00 (0.00)		0.16 (0.06)	0.50 (0.12)	
Early-spawned (17 Aug. 1983) (59 full-sib families)						
Sib analyses						
h_s^2	0.60 (0.39)	0.65 (0.41)	0.26 (0.27)	0.24 (0.27)	0.38 (0.28)	0.52 (0.32)
95% LCL h_s^2	0.61	0.56	0.08	0.00	0.06	0.23
h_d^2	0.65 (0.38)	0.66 (0.38)	0.25 (0.31)	0.48 (0.14)	0.07 (0.30)	0.21 (0.31)
h_{sd}^2	0.65 (0.24)	0.65 (0.24)	0.25 (0.17)	0.09 (0.08)	0.22 (0.18)	0.37 (0.20)
Offspring–parent regression						
Offspring–sires	0.16 (0.07)	0.00 (0.00)		0.00 (0.10)	0.00 (0.00)	
Offspring–dam	0.03 (0.13)	0.00 (0.00)		0.48 (0.14)	0.00 (0.00)	
Late-spawned (20 Sept. 1983) (59 full-sib families)						
Sib analyses						
h_s^2	0.85 (0.37)	0.58 (0.31)	0.11 (0.21)	0.29 (0.36)	0.33 (0.28)	0.06 (0.29)
95% LCL h_s^2	0.44	0.67	0.04	0.09	0.3	0.03
h_d^2	0.42 (0.25)	0.40 (0.26)	0.31 (0.27)	0.73 (0.43)	0.21 (0.32)	0.54 (0.41)
h_{sd}^2	0.63 (0.20)	0.49 (0.18)	0.21 (0.14)	0.51 (0.24)	0.27 (0.19)	0.30 (0.21)
Offspring–parent regression						
Offspring–sires	0.18 (0.20)	0.00 (0.00)		0.00 (0.14)	0.00 (0.0)	
Offspring–dam	0.54 (0.24)	0.00 (0.00)		0.10 (0.16)	0.00 (0.0)	

both early- and late-spawned fish are analyzed together. This regression analysis would be expected to produce higher estimates of heritability because the contrast between early- and late-spawned fish contributes to the slope of the regression. In our analysis of variance, much of that contrast is modelled as the effect of spawning date on size. While some of the effect of spawning date may reflect genetic variability, it could not be analyzed because early spawners could not be mated with late spawners. The lesser estimates from parent–offspring regression may be a consequence of interannual variation of environmental conditions in the North Pacific Ocean and of interactions or correlations of genotypes and environment: some genotypes may achieve larger size than others in one brood year but not in another. Parents and their offspring, being from different brood years, would experience different conditions.

Under an additive model of selection, genetic variation of traits related to fitness is reduced by selection (Fisher 1958; Crow 1986, Ch. 4). Male size is probably related to fitness in pink salmon as it is in other salmonids. Apart from any dependency of survival on size, male size is correlated with fitness through mating success (Noltie 1989). Such an additive model is not consistent with the large phenotypic variability of male size that we and others have observed (Fig. 1; Beacham and Murray 1985; Noltie 1989) and is not consistent with high heritability of male size.

The relatively great additive genetic variation of male size in pink salmon may be a consequence of nonadditive genetic variation, genotype–environment interactions, or correlations of genotypes and environments. Whatever the mechanism the relatively great genetic variability of size in pink salmon males suggests that the variability is conserved as part of a stable breeding strategy in natural populations and is important to population fitness (Gross 1985; Noltie 1989). In females, size variation is predictably less than in males, because of high correlations between body size and fecundity, which is directly related to fitness.

High correlations between length and weight are expected and have been observed in other salmonids (e.g., Gjerde and Gjedrem 1984). Length is measured more precisely in our data (nearest millimetre vs. nearest 100 g; <1% vs. <10%) and is less affected by changes in body shape and water content associated with sexual maturation and anadromous migration. The lesser values of heritability of weight and

TABLE 3. Genetic (G), environmental (E), and phenotypic (P) correlations (r) based on combined sire and dam effects between length and weight and between length and day of return from the sea to the natal stream for Auke Creek Pink salmon. Standard errors are given in parentheses. See Table 2 for further information.

	Males		Females	
	Length and weight	Length and day	Length and weight	Length and day
	Full model includes spawning dates as main effect (118 full-sib families)			
r_G	0.95 (0.02)	0.40 (0.17)	1.01 (0.06)	0.45 (0.22)
r_E	0.79 (0.06)	0.13 (0.10)	0.72 (0.04)	−0.10 (0.10)
r_P	0.88 (0.01)	0.22 (0.05)	0.80 (0.02)	0.06 (0.06)
	Early-spawned (17 Aug. 1983) (59 full-sib families)			
r_G	0.95 (0.03)	0.36 (0.24)	1.07 (0.12)	0.62 (0.36)
r_E	0.76 (0.08)	0.2 (0.15)	0.70 (0.05)	−0.10 (0.13)
r_P	0.88 (0.02)	0.25 (0.07)	0.78 (0.04)	0.12 (0.09)
	Late-spawned (20 Sept. 1983) (59 full-sib families)			
r_G	0.96 (0.02)	0.45 (0.23)	0.98 (0.06)	0.31 (0.29)
r_E	0.82 (0.07)	0.06 (0.13)	0.80 (0.04)	−0.30 (0.16)
r_P	0.89 (0.02)	0.20 (0.07)	0.84 (0.03)	−0.10 (0.09)

the greater standard errors (Table 2) are probably a consequence of additional sources of variation that affect weight measurements.

Genetic, environmental, and phenotypic correlations between size and date of spawning reported for domesticated rainbow trout ($r_G = 0.48$, $r_E = 0.07$, $r_E = 0.12$; Huang and Gall 1990) are similar to our observed correlations between length and date of return. Crandell and Gall (1993), however, report higher correlation estimates in rainbow trout ($r_G = 0.6$, $r_P = 0.5$).

High heritability of body size has consequences for harvest management. Genetic response to size selection by gillnet fishing has been proposed as an explanation of secular declines of individual size in pink salmon fisheries (Ricker 1981), and McAllister and Peterman (1992) propose an experiment to test the hypothesis that artificial selection in pink salmon fisheries can change individual body size. That heritability of male body size is high suggests that size selection in pink salmon fisheries would indeed be detrimental to the productivity of the fishery if large fish were removed from the breeding population. We note, however, that heritability of size is less when estimated from offspring–parent regression, which we take to be a consequence of interannual variability of environment. This suggests that the genotypes producing the largest phenotypes in one year do not necessarily produce the largest phenotypes in another year and that response to directional selection by a size-selective fishery would not be as rapid as predicted by high estimates of heritability of size within a single generation. Even offspring–parent regression may overestimate effective heritability over several generations; climate, including temperature of the pink salmon's epipelagic habitat, cycles over periods of decades (Royer 1993), which suggests that parents and offspring share more similar environments than do relatives several generations apart.

The substantial genetic correlation of size and timing of return that we observe also could have consequences for harvest management. Alexandersdottir (1987) reviewed evidence that selection with respect to timing has had detrimental effects on pink salmon productivity in some fisheries. Our data suggest that selection against later returning fish could have the correlated effect of reducing the individual size of fish in the population. Again, however, effects of interannual environmental variation do not enter into our estimates of correlation and might slow or eliminate correlated responses of size to selection on timing.

High heritability of size has consequences for broodstock management in ocean ranching. Gjedrem (1986) anticipated progress from artificial selection in salmon ocean ranching and Jonasson (1993) reports on progress in developing a program of selection for increased size and other characters in ocean-ranched Atlantic salmon. Our observation of high heritability of size in anadromous pink salmon, which are also produced by ocean ranching, suggests that rapid progress could be attained from a selection program.

Again, however, the discrepancy between our estimates of heritability of size from intraclass correlations and from offspring parent regression suggests caution in predicting progress. Response to directional selection may not be as rapid as predicted by our high estimates of heritability because environmental conditions vary from year to year. One attempt at individual selection for increased size in replicate lines sampled from a large pink salmon hatchery did not result in a demonstrable response after one generation (Russell 1992). A similar result was reported by McIntyre et al. (1988) in a four-generation family selection program in which survival of hatchery (ocean-ranched) coho salmon was the subject trait. Despite significant increase after two generations, progress was not sustained in succeeding generations presumably because of varying environmental conditions or losses of fitness due to inbreeding in the selected lines.

Variability of size is high in Auke Creek pink salmon, and Beacham and Murray's (1988) similar result from analysis of captive pink salmon from a different stock suggests that this substantial variability may be characteristic of many populations. Variation of size is probably important to population fitness in pink salmon. Response to size selection exercised by a fishery or an ocean-ranching program may not be rapid or even immediately discernible, however,

because of interannual variations of environment, interactions and correlations of genotypes and environment, covariances of size with other traits, and relatively low selection intensity of pink salmon harvest. Even so, resource managers should recognize the importance of size variation to population fitness, especially in wild-spawning stocks, and use strategies that conserve size variation.

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APPENDIX. Analyses of variance of lengths and weights of male and female pink salmon in full-sib – half-sib families. Computed by GLM procedure in PC SAS version 6.04

Source	df	Type III sum of squares	Mean square
		Length of males	
Spawn date	1	23 524.7429	23 524.7429
Sire (spawn date)	57	156 078.6185	2 738.2214
Dam (spawn date × sire)	48	58 491.9886	1 218.5831
Error	534	320 535.5469	600.2538
Corrected total	640	587 766.0624	
		Weight of males	
Spawn date	1	2.076 957 59	2.076 957 59
Sire (spawn date)	57	15.851 503 21	0.278 096 55
Dam (spawn date × sire)	48	6.696 644 97	0.139 513 44
Error	534	38.961 337 91	0.072 961 31
Corrected total	640	66.890 388 77	
		Length of females	
Spawn date	1	18 413.478 53	18 413.478 53
Sire (spawn date)	57	51 467.482 61	902.938 29
Dam (spawn date × sire)	50	33 394.332 25	667.886 65
Error	394	180 153.215 9	457.241 7
Corrected total	502	301 117.952 3	
		Weight of females	
Spawn date	1	1.394 076 51	1.394 076 51
Sire (spawn date)	57	4.976 041 04	0.087 298 97
Dam (spawn date × sire)	50	2.693 036 38	0.053 860 73
Error	394	18.332 228 79	0.046 528 50
Corrected total	502	29.329 855 27	

Viability and Freshwater Performance of Atlantic Salmon (*Salmo salar*) × Brown Trout (*Salmo trutta*) Triploid Hybrids

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Survival to initiation of feeding and early freshwater growth were measured for both diploid (2N) and triploid (3N) Atlantic salmon (*Salmo salar*) and 2N and 3N hybrids between female Atlantic salmon and male brown trout (*Salmo trutta*) to evaluate the triploid hybrid's potential for commercial culture. Crosses were made in 1990 and 1991 and triploidy was induced by heat shock. Average survival to initiation of feeding generally did not differ among the 2N Atlantic salmon and hybrids within years, nor between the 3N crosses within years. Average survival to initiation of feeding of the 3N Atlantic salmon and hybrids relative to the corresponding 2N crosses was reduced by 2 and 9%, respectively, in 1990, and by 55 and 62%, respectively, in 1991. Five freshwater growth trials were conducted to compare the different crosses. Results indicated no consistent differences in relative growth rates, although the hybrids demonstrated greater variability in growth at age 0+.

La survie jusqu'à l'initiation de l'alimentation et la croissance précoce en eau douce ont été mesurées chez des saumons atlantiques (*Salmo salar*) diploïdes (2N) et triploïdes (3N) ainsi que chez des hybrides 2N et 3N de saumons atlantiques femelles et de truites brunes (*Salmo trutta*) mâles afin d'évaluer le potentiel des hybrides triploïdes pour la culture commerciale. Les croisements ont été réalisés en 1990 et 1991 et la triploïdie a été induite par choc thermique. De façon générale, la survie moyenne jusqu'à l'initiation de l'alimentation n'a pas été différente entre les saumons atlantiques 2N et les hybrides d'une même année, ni entre les croisements 3N d'une même année. La survie moyenne jusqu'à l'initiation de l'alimentation chez les saumons atlantiques 3N et les hybrides 3N par rapport aux croisements 2N a été réduite de 2 et 9 %, respectivement, en 1990, et de 55 et 62 %, respectivement, en 1991. Cinq essais de croissance en eau douce ont été réalisés pour comparer les différents croisements. Les résultats n'ont indiqué aucune différence uniforme dans les taux de croissance relatifs, bien que les hybrides aient présenté une plus grande variabilité au niveau de leur croissance à l'âge 0+.

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Commercial culture of salmonids is constrained by two problems related to their sexual maturity: loss of market value of the fish associated with maturity, and legal restrictions on the culture of fertile fish where escape into natural water bodies is a possibility. Sexual maturation in salmonids is accompanied by development of secondary sexual characteristics, which include a darkening of skin color and a deterioration in the flavor and texture of the flesh. These changes dramatically reduce the market value of the fish. To maintain the high value of their product, salmonid growers must limit their production period and market their fish prior to the normal age of sexual maturation. Also, a variable proportion of the fish undergo sexual maturation one or more years earlier than normal. These fish must either be identified and harvested early or culled at the time of harvest. In either case, a significant financial loss may be incurred (Hunter and Donaldson 1983; Lincoln and Scott 1983; Solar et al. 1984b; Bye and Lincoln 1986; Friars and Benfey 1991).

The growing concern worldwide for conservation of indigenous species has included critical review of the effects of reproduction of escaped farmed fish in the wild and of their interbreeding with natural stocks (Hindar et al. 1991; Pepper 1991; Saunders 1991). The incidence of escaped pen fish interbreeding with natural runs of Atlantic salmon (At; *Salmo salar*) in Norway is of particular concern (Bergan et al. 1991; Gausen and Moen 1991; Lura and Sægrov 1991; Mork 1991; Gausen 1993). The farming of fertile rainbow trout (*Oncorhynchus mykiss*) in the Canadian provinces of Newfoundland and Labrador, where rainbow trout has not been introduced, is banned by law. Also, import of non-indigenous stocks of fertile Atlantic salmon for commercial culture in Newfoundland is discouraged by federal authorities (Sutterlin and Collier 1991). The potential for escape of farmed fish has been used by the State of Alaska as an argument supporting the state-wide ban of net-pen aquaculture (R. Painter, Executive Director, Alaska Mariculture Association (disbanded), 130 Seward Street, Suite 504, Juneau,

AK, 99801, personal communication). Atlantic salmon have been caught in the commercial offshore fishery in southeastern Alaska. These fish were presumed to be escapees from net-pen farms in British Columbia (Wing et al. 1992). In addition, Wing et al. (1992) relate reports of mature Atlantic salmon migrating up freshwater streams in British Columbia.

Sterilization has been proposed to counter these problems. Three methods to induce sterility in salmonids include hormonal treatment, triploidization, and hybridization. Sterilization can be performed by immersion of eggs or alevins in a bath of steroid hormones and/or by feeding of fry with hormone-treated feed (see reviews by Donaldson and Benfey 1987; Donaldson et al. 1991). However, sale of hormone-treated fish for human consumption is illegal in the United States and Canada.

Sterility can also be achieved by triploidization. Triploidy can be induced through retention of the second polar body in a fertilized egg resulting in a fish with three functional sets of chromosomes. Triploidy occurs naturally at a very low frequency in salmonids, but can be induced artificially by application of a temperature or pressure shock shortly after fertilization. Triploid salmonids are highly viable and indistinguishable in appearance from diploids (see review by Ihssen et al. 1990). Although triploids of both sexes are functionally sterile, gonadal development is only arrested in the female. Male triploids produce motile aneuploid sperm (Allen et al. 1986; Benfey et al. 1986) and develop secondary sexual characteristics comparable to diploid males (see review by Ihssen et al. 1990). Consequently, production of all-female triploid fish would be advantageous for commercial culture. Commercially feasible schemes for the producing sex-reversed XX male broodstock, which can be used to produce all-female progeny in combination with induced triploidy, are presented by Thorgaard (1983) and Donaldson and Benfey (1987).

Artificial hybridization has been performed among a wide variety of salmonid species for the purpose of combining favorable characteristics from the parental species. A lack of gonad development or gametic sterility has been observed in many of these hybrids, but survival and growth are typically poor (see reviews by Chevassus 1979; 1983). However, the viability of triploid hybrids is often increased and this has renewed interest in investigating the aquaculture potential of salmonid hybrids (Chevassus et al. 1983; Scheerer and Thorgaard 1983; Arai 1986; Seeb et al. 1988, 1993; Gray et al. 1993).

Although triploidization and hybridization can both be nearly 100% effective, absolute effectiveness cannot be assured for either treatment. In combination, however, they can produce fish with a much greater certainty of sterility. Similarly, Donaldson et al. (1991) proposed application of hormonal sterilization treatments of triploid salmonids should triploidization alone be considered an insufficient guarantee of sterility.

Atlantic salmon are the most extensively cultured salmonids in net pens worldwide. The fertility and performance of triploid interspecific hybrids involving Atlantic salmon are thus of commercial interest. If such a diploid hybrid is sterile and performance of the diploid or triploid hybrid comparable to that of Atlantic salmon, it could provide a viable alternative species for net-pen culture. Also, there is the possibility that the hybrid will show enhancement for some culture characteristics.

The most likely candidate for hybridization with Atlantic salmon is the brown trout (Bn; *Salmo trutta*). They are closely related to Atlantic salmon and natural hybridization between the two species has been widely reported (Payne et al. 1972; Solomon and Child 1978; Beland and Roberts 1981; Verspoor 1988; Garcia de Leaniz and Verspoor 1989; Hurrell and Price 1991; Jansson et al. 1991; McGowan and Davidson 1992a; Youngson et al. 1992). Both reciprocal crosses between the two species show high viability (Refstie and Gjedrem 1975; Chevassus 1983; McGowan and Davidson 1992b; Gray et al. 1993), although the Atlantic salmon female × brown trout (At × Bn) male cross appears more viable, with little if any reduction in viability relative to the parental species (see review by McGowan and Davidson 1992b). Unlike observations in many triploid salmonid hybrids, Gray et al. (1993) reported that survival to initiation of feeding of the triploid At × Bn and Bn × At hybrids was reduced from that for the diploid hybrids. Survival and growth for 2N At × Bn male hybrids was reported to be reduced relative to the 2N parental species (Refstie and Gjedrem 1975; Refstie 1983). No reports on the growth performance of 3N hybrids are available.

There have been some reports in the literature regarding production of viable F_2 and backcross progeny from F_1 At × Bn and Bn × At hybrids. However, some of these reports have been shown to be erroneous and others indicate that effective introgression of genomes of the two species is unlikely (Nyman 1970; Nygren et al. 1975; Johnson and Wright 1986).

A second candidate species for hybridization with Atlantic salmon is brook trout (Bk; *Salvelinus fontinalis*) in light of the success observed in hybridization between brook trout and brown trout, often referred to as tiger trout. Both reciprocal diploid hybrids are sterile and show relatively high viability compared with many other interspecific hybrids, with the female brown trout × male brook trout cross showing greater survival to initiation of feeding (Chevassus 1979; Blanc and Chevassus 1986; McKay et al. 1992a). Triploidy increases the hybrid's viability (Scheerer and Thorgaard 1983; Scheerer et al. 1987), and growth of triploid tiger trout is comparable to or greater than growth of the diploid hybrid (McKay et al. 1992b).

We have measured the viability of 2N and 3N hybrids between female Atlantic salmon and male brown trout or brook trout relative to control 2N and 3N Atlantic salmon. Survival and growth in fresh water were also measured for these crosses.

Materials and Methods

Diploid and triploid Atlantic salmon and hybrids between female Atlantic salmon and male brown trout or brook trout were produced in 1990 and 1991 at Washington State University (WSU). Data on survival to initiation of feeding were recorded for each mating within each cross. The fish from both year-classes were placed in an early (age 0+) then a late (age 1+) freshwater growth trial. In 1991, 2N Atlantic salmon and 3N At × Bn hybrids were also produced at Stolt Sea Farms, Port Angeles, Wash. At the eyed stage, the eggs were transferred to the Stolt Sea Farms, Rochester, Wash., freshwater hatchery. The fish from these crosses were placed in an early (0+) growth trial. Details on the production of the crosses and the growth trials are given below.

WSU 1990 Year-class

In 1990 at WSU, eggs from four Atlantic salmon females were divided into 12 sublots and each sublot was fertilized with sperm from one of four males each of Atlantic salmon, brown trout, or brook trout. Atlantic salmon gametes were obtained from Cascade Aqua Farms, Winlock, Wash., and brown and brook trout sperm from Ford Trout Hatchery of the Washington Department of Wildlife, Ford, Wash. Triploidy was induced by immersing one half of the eggs (approximately 300) from each sublot for 10 min in a 29°C water bath beginning at 10 min postfertilization (Chourrout 1980; Scheerer and Thorgaard 1983). The eggs were incubated in separate containers in a Heath™ stack incubator. Water temperature through initiation of feeding averaged 9°C. Percent survival to the eyed, hatch and initiation of feeding stages was calculated for each single-pair mating. Statistical analyses between ploidy levels within crosses were performed with paired *t*-tests and between crosses of the same ploidy with independent *t*-tests. (All analyses of percentage values in this study involve arcsine transformation of the data, and all statements of statistical significance assume a probability value of less than 0.05.)

Success of triploidy induction was determined by flow cytometric analysis of blood samples following a modification of the procedure described by Allen (1983). Blood samples from 58 one-month-old fry were taken from both the Atlantic salmon and At × Bn triploid crosses, and from 28 fish from the At × Bk triploid cross. Survival to initiation of feeding of the 2N and 3N At × Bk hybrids was so poor that experimentation with these crosses was discontinued.

The surviving At × At and At × Bn fry were raised in fresh water and placed in two different growth trials. Following initiation of feeding 100 randomly chosen fish from each of the four matings within the At × At and At × Bn crosses were pooled (with the exception of one of the At × Bn crosses, where only seventy-one 2N and ninety-six 3N hybrids were added to the pools, owing to low survival). The fish were raised for 6.5 mo, then the largest and smallest 20% of the fish in each cross/ploidy group, as measured by length, were culled. Forty fish from each group were stocked in four randomly assigned plastic grow-out containers (16.3-L water volume plastic buckets), 10 fish per container, 4 containers per cross/ploidy group, within the same recirculating system.

The fish were raised for 10 wk. Weight samplings (total number and weight) were performed every 2 wk for each container. Water temperature was 14.4 ± 0.3°C. The daily light regime was 14 h light : 10 h dark. The fish were fed a commercial diet at approximately 1.2 times recommended rates (Ewos Canada Ltd., Feed Rate Table; Feed Type: Vextra Starter and Smolt; Species: Atlantic and chinook salmon), which provided feed to excess or to near excess. Feeding rates were adjusted weekly. The daily feed ration was delivered by an automatic feeder over an 8- to 10-h period. The occasional mortalities that occurred during the growth trial were replaced by fish of similar size from a stock of extra fish, to maintain equal densities.

The coefficient of variation for weight was calculated for each grow-out container at the beginning and end of the experiment. The differences between the initial and final coefficients of variation were calculated and averaged within crosses. Average differences were compared among crosses with a one-way analysis of variance followed by multiple pairwise comparisons using Scheffé's test. Growth rates were calculated for each grow-out container as the slope of the linear regression line fitted to the plot of the natural logarithm of the average weight of the fish in each container and the day of sampling. The values were then averaged within each cross and compared between crosses with a one-way analysis of variance, followed by multiple pairwise comparisons using Scheffé's test.

After the early growth trial, the fish were transferred to larger rearing tanks and raised to 18 mo (age 1+) after initiation of feeding, at which time they were placed in a second growth trial. Thirty At × At (2N), 18 At × At (3N), and 31 At × Bn (3N) fish were individually PIT (passive integrated transponder) tagged and weighed, and reared in separate 1.1-m^3 tanks in the same recirculating system for 71 d. Feeding procedures and light regime were the same as for the early growth trial. The biweekly samplings involved weighing all fish individually. Daily water temperature averaged 15°C. Ploidy was confirmed for all fish after the growth trial, and data for diploid fish detected among the triploid crosses were not used in the subsequent analyses. Growth rates were calculated for each fish individually, then averaged within crosses. Average growth rates were compared between crosses as described for the 1990 early growth trial. This analysis necessarily presumes no unrelated tank effects; the three tanks were identical in construction and lighting, and were located within the same recirculating system with equal inflow rates.

WSU 1991 Year-class

In 1991, 2N and 3N fish were produced at WSU by crossing sublots of the pooled eggs from nine At females with the unpooled sperm from eight and seven males each of At or Bn, respectively. There were approximately 150 eggs per Atlantic salmon cross/ploidy mating, and 250 eggs per At × Bn hybrid cross/ploidy mating. The Atlantic salmon gametes were obtained from Stolt Sea Farms, Port Angeles, Wash., and the brown trout sperm from Ford Trout Hatchery of the Washington Department of Wildlife, Ford, Wash. Incubation temperature averaged 8.5°C. Survival data through the different stages of incubation were recorded and analyzed with independent *t*-tests as described for the 1990 crosses. Crosses made with one male from both the Atlantic salmon and the brown trout showed no survival to the eyed stage, and data for these matings were not used in the analyses.

Following initiation of feeding (which occurred concurrently for the 2N and 3N fish within crosses, but 11 d earlier for the At × Bn than for the At × At) all surviving fish from the different matings within each cross (range = 0–132 fish) were pooled and stocked evenly among three randomly assigned grow-out containers per cross/ploidy group. The fish were raised in the same containers, with similar grow-out and feeding conditions as used in the WSU 1990 year-class early growth trial. At 30 d after initiation of feeding, stocking density was reduced to 50 randomly chosen fish per container. The number of surviving fish was calculated at day 60 and day 90. Survival from 30 to 90 d after initiation of feeding was analyzed with a one-way analysis of variance followed by multiple pairwise comparisons using Scheffé's test.

At approximately 5 mo after initiation of feeding the fish were pooled within cross/ploidy groups and the largest and smallest 10% were culled. From the remaining fish, 60 per cross/ploidy group were randomly restocked in the grow-out

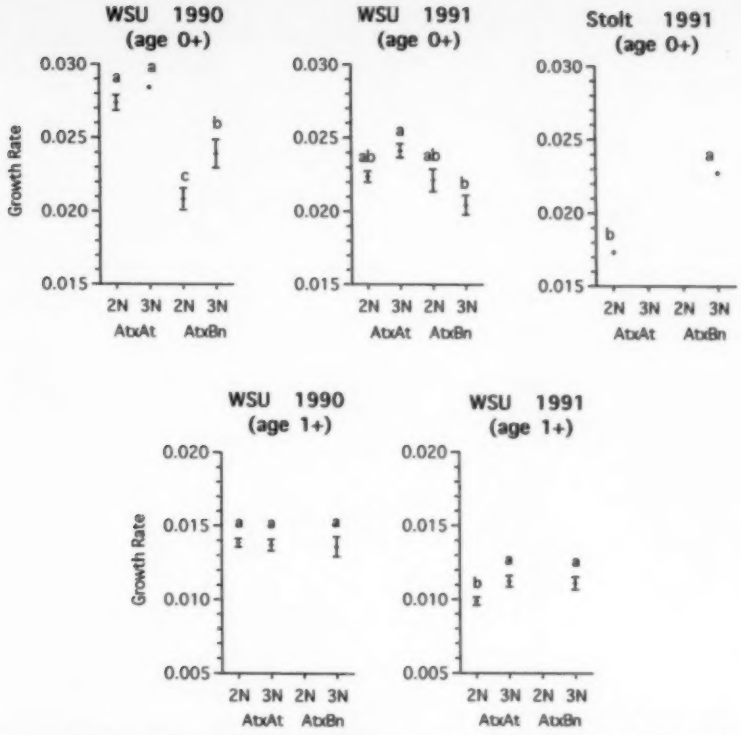

FIG. 1. Average growth rates (±1 SE) for the early (age 0+) and late (age 1+) freshwater growth trials performed at Washington State University (WSU) with fish from the 1990 and 1991 year-classes, and for the early freshwater growth trial performed at Stolt Sea Farms, Rochester, Wash., with fish from the 1991 year-class. Multiple pairwise comparisons were performed with Scheffé's test ($\alpha = 0.05$); significant differences are indicated by different letters.

containers at a density of 15 fish per container, with four randomly assigned containers per cross. An early growth trial was performed with these fish and a statistical analysis of the data in a manner identical to that described for the previous year's early (age 0+) trial.

Following the growth trial the fish were transferred to the 1.1-m^3 tanks described for the 1990 year-class late freshwater growth trial. At 11 mo after initiation of feeding the remaining 46 At × At(2N), 54 At × At(3N), and 52 At × Bn (3N) fish were placed in a late (age 1+) growth trial for 70 d. The experiment was conducted in the same manner as for the 1990 age 1+ fish, except that water temperature averaged 11.5°C. Ploidy was determined for all fish at the end of the trial to determine success of triploidy induction and to identify diploid fish among the triploid crosses.

Stolt 1991 Year-class

In 1991, At × At (2N) and At × Bn (3N) crosses were also produced at Stolt Sea Farms, Port Angeles, Wash., a major commercial producer of Atlantic salmon. The crosses were made with sublots of pooled eggs and unpooled sperm from the same brood fish used for the WSU 1991 crosses. Following fertilization the eggs were pooled within crosses. At the eyed stage the eggs were transferred to the Stolt Sea Farms freshwater hatchery in Rochester, Wash. Data for survival during incubation were not collected for these fish.

At 3.5 mo after initiation of feeding, three 0.19 m^3 fiberglass tanks per cross were stocked at a density of 97 fish/tank. The six tanks were assigned alternately to one cross or the other. A growth trial was conducted for 127 days. The fish were monitored and cared for and the data analyzed as described for the early growth experiments performed at WSU, except that water temperature was constant at 10.5°C, and the light regime was constant at 24 h light.

TABLE 1. Percent triploidy as determined by flow cytometric analysis and number of fish examined for the 1990 and 1991 Washington State University (WSU) and the 1991 Stoltz Sea Farms year-classes.

Cross	Percent triploidy and number of fish examined (in parentheses)		
	WSU 1990	WSU 1991	Stolt 1991
At × At (2N)	5% (37)	0% (48)	—
At × At (3N)	93% (58)	100% (57)	—
At × Bn (2N)	0% (39)	—	—
At × Bn (3N)	92% (58)	100% (53)	99% (171)
At × Bk (2N)	—	—	—
At × Bk (3N)	96% (28)	—	—

Results

WSU 1990 Year-class

The average percent success of triploidization was 93% for the At × At (3N) matings and 92% for the At × Bn (3N)

TABLE 2. Average percent survival from fertilization to the eyed, hatch, and initiation of feeding stages of incubation for the 1990 and 1991 Washington State University (WSU) year-classes.

Cross	WSU 1990 year-class			WSU 1991 year-class		
	Eyed	Hatch	Init. of feeding	Eyed	Hatch	Init. of feeding
At × At (2N)	67	61	57	52	39	29
At × At (3N)	65	59	56	34	15	13
At × Bn (2N)	68	64	63	52	41	37
At × Bn (3N)	61	56	54	24	15	14
At × Bk (2N)	53	24	1			
At × Bk (3N)	56	42	8			

TABLE 3. Initial and final average weights (g/fish) and coefficients of variation (%; in parentheses) for the early (age 0+) and late (age 1+) freshwater growth trials performed with fish from the 1990 or 1991 year-classes at Washington State University (WSU), and for the early (age 0+) freshwater growth trial performed with fish from the 1991 year-class at Stolt Sea Farms, Rochester, Wash.

Growth trial	Atlantic salmon				At × Bn			
	2N		3N		2N		3N	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
WSU 1990 early, age 0+	5.5 (32)	34.2 (38)	5.3 (25)	35.2 (29)	8.0 (33)	31.8 (70)	7.4 (37)	36.1 (65)
WSU 1991 early, age 0+	6.8 (36)	32.7 (48)	7.1 (29)	38.8 (47)	6.1 (42)	27.8 (68)	6.6 (30)	28.0 (64)
Stolt 1991 early, age 0+	1.2	16.2 (45)	—	—	—	—	1.7	42.9 (86)
WSU 1990 late, age 1+	101 (23)	273 (20)	91 (19)	202 (18)	—	—	111 (37)	311 (31)
WSU 1991 late, age 1+	88 (27)	174 (26)	80 (34)	171 (25)	—	—	79 (61)	169 (55)

matings (Table 1). The small number of 3N fish found among the 2N crosses was due either to accidental mixing of the crosses or a low level of natural triploidization.

Survival of the 3N Atlantic salmon and the 2N and 3N At × Bn hybrids was similar to or slightly reduced from that of the 2N Atlantic salmon during both the incubation and freshwater grow-out periods; however, survival of the At × Bk crosses was very low (Table 2). During the early freshwater growth trial eight fish from four different grow-out containers died; four jumped out of the containers, and four were injured by eye pecking. Only a single mortality, due to jumping, was recorded in the late-growth trial.

Initial and final average weights and corresponding coefficients of variation for each cross/ploidy group in both the early and late growth trials are presented in Table 3. Coefficient of variation for weight increased during the early growth experiment for all crosses. The average increase was not statistically different between ploidy levels within crosses. However, the At × Bn hybrids showed a significantly greater increase than did Atlantic salmon of the same ploidy. Coefficient of variation for weight decreased during the late growth trial for all cross/ploidy groups; statistical comparisons were not possible.

Average growth rates of the 2N and 3N Atlantic salmon in the early growth trial were not significantly different from each other and were greater than those of the At × Bn (3N), which in turn was greater than those of the At × Bn (2N) (Fig. 1). In the late growth trial, average growth rates of the At × At (2N), At × At (3N), and At × Bn (3N) were not significantly different (Fig. 1).

Calculating growth rate as described in Materials and Methods assumes that growth during the experimental period was exponential. Correlation coefficients for the regression lines calculated for each replicate tank in the three different early freshwater growth trials were all greater than 0.98. Correlation coefficients for individual fish in the two late freshwater growth trials were all greater than 0.95, with two exceptions ($r = 0.90$ and 0.94), and average values per cross within a year-class were all greater than 0.98. These high correlation coefficient values justify the assumption of exponential growth during these trials.

WSU 1991 Year-class

The average percent success of triploidization as measured in the fish at the end of the late growth trial was 100% for both the At × At (3N) and At × Bn (3N) crosses (Table 1).

Survival during incubation was low for both the 2N Atlantic salmon and At × Bn hybrids, and much lower for the 3N crosses (Table 2). However, survival during freshwater grow-out was high. Average survival of the fish during the period from 30 to 90 d after initiation of feeding was 76% for the At × At (2N), 84% for the At × At (3N), 90% for the At × Bn (2N), and 85% for the At × Bn (3N). These values were not significantly different. Eleven fish from eight different grow-out containers died during the early growth trial, nine due to jumping, one due to eye pecking, and one for undetermined reasons Seven fish from two tanks died during the late grow-out trial, most of which showed signs of infection in the dorsal area where the PIT tags were implanted.

Initial and final average weights and coefficients of variation for each cross in both the early and late growth trials are presented in Table 3. As for the 1990 year-class, the average increase in coefficient of variation for weight during the early growth experiment showed no statistical differences between ploidy levels within crosses, but was greater for the 3N crosses than for the respective 2N crosses. Coefficient of variation decreased slightly for all crosses in the late growth trial, as for the 1990 year-class.

Average growth rates in the early trial did not differ significantly between cross/ploidy groups, with the exception that the At × At (3N) grew faster than the At × Bn (2N) (p = 0.007). In the late growth trial, the average growth rates of the 3N At × At and At × Bn were similar, and both were greater than those of the At × At (2N) (p = 0.004 and 0.029, respectively) (Fig. 1).

Stolt 1991 Year-class

Survival rates in the replicate tanks for each cross in the early growth trial performed at Stolt Sea Farms, Rochester, Wash., were 78, 73, and 63% (average = 71%) for the At × At (2N) and 77, 84, and 87% (average = 82%) for the At × Bn (3N). Average survival values were not significantly different. Mortality was due primarily to jumping out of the uncovered grow-out tanks during the final month of the trial.

Initial and final average weights and coefficients of variation for the crosses in this trial are presented in Table 3. The average growth rate of the At × Bn (3N) was significantly greater than that of the At × At (2N) (p = 0.003) (Fig. 1).

Discussion

Survival of Triploids and Hybrids

Survival to initiation of feeding of the 3N At × At and At × Bn crosses relative to their respective 2N crosses was reduced by 2 and 9%, respectively, in 1990, and by 55 and 62%, respectively, in 1991. These differences were all statistically significant except for the 1991 year-class Atlantic salmon. Mortality during incubation is typically higher for triploids, presumably due to the deleterious effects of the shocks used to induce second polar body retention (see review by Ihssen et al. 1990). The much larger reduction observed in the 1991 crosses was probably due to poor egg quality. The eggs were overripe and average survival to initiation of feeding of the At × At (2N) controls was only 29%. A relative increase in mortality rate of triploids due to poor egg quality has been reported previously (Lou and Purdom 1984; Johnstone 1985; Happe et al. 1988), and has no doubt contributed to the wide variability in success of triploidy induction and early survival of triploids reported for different heat- and pressure-shock protocols.

Within each of the two WSU year-classes, survival of the 2N and 3N At × Bn hybrids was not significantly different from that of Atlantic salmon of the same ploidy, except that the 1991 At × Bn (3N) showed higher survival than the At × At (3N) (p = 0.016). These results are similar to those reported for 2N At × Bn hybrids relative to the parental diploids by Refstie and Gjedrem (1975), Chevassus (1979), McGowan and Davidson (1992b), and Gray et al. (1993).

In the 1990 WSU experiment, average survival to initiation of feeding of the At × Bk 2N and 3N hybrids was 1 and 8%, respectively. Previous reports also indicate that the diploid At × Bk hybrid has very low viability (Blanc and Chevassus 1979; Gray et al. 1993). Gray et al. (1993) produced both 2N and 3N At × Bk hybrids but found that survival of the 3N hybrids was not significantly increased over that of the 2N hybrids (approximately 2 and 1%, respectively). However, survival of their Atlantic salmon controls was poor (17% to initiation of feeding) and it was thought that this may have unduly influenced the relative survival of the hybrids. In our experiments viability of the 3N At × Bk hybrids was significantly greater than that of the diploids (p = 0.031), but was nonetheless too low to be of commercial interest.

No significant differences in survival were observed during freshwater rearing from 30 and to 90 d after initiation of feeding between the At × At (2N), At × At (3N), At × Bn (2N), and At × Bn (3N) crosses of the WSU 1991 year-class. Survival during the different early and late growth trials was high, except for the Stolt early growth trial. Mortality in the five trials was primarily due to jumping and was unrelated to differential viability of the crosses. Similarity of survival in freshwater for these crosses is also reported by Refstie and Gjedrem (1975), Chevassus (1979), and McGowan and Davidson (1992b). Survival in fresh water is, therefore, not adversely affected by either triploidization or hybridization.

Growth of Triploids and Hybrids

Comparisons of growth of 2N and 3N fish within crosses varied among our experiments. Growth rate of 2N and 3N Atlantic salmon was not significantly different in four of the five trials; in the WSU 1991 year-class late growth trial, the At × At (3N) grew significantly faster than the At × At (2N). Comparisons between 2N and 3N At × Bn hybrids in the two WSU early growth trials demonstrated that growth of the 3N hybrids was greater than that for the 2N hybrids from the 1990 year-class, and that their growth was similar in 1991 year-class. Triploidization of salmonids has been reported to depress growth in some studies (Thorgaard et al. 1982; Utter et al. 1983; Solar et al. 1984a), but either no depression or occasionally an enhancement of growth was observed in other studies (Benfey and Sutterlin 1984; Johnson et al. 1986; Guo et al. 1990; Quillet and Gaignon 1990; McKay et al. 1992b).

Results from the four WSU growth trials indicated that variability in growth rate is similar within ploidy levels. No observations relative to variability of growth between 2N and 3N salmonids were found in the published literature.

The relative growth rates of Atlantic salmon (2N and 3N) and At × Bn hybrids (2N and 3N) varied among our experiments. In the early growth trials performed at WSU, the 2N and 3N Atlantic salmon generally outgrew the At × Bn hybrids. However, the At × Bn (3N) dramatically outgrew the At × At (2N) in the trial performed at Stolt Sea Farms ($p = 0.003$). In the WSU late freshwater trials, growth did not differ between the At × At (2N), At × At (3N), and At × Bn (3N) for the 1990 year-class, and growth of the At × At (2N) was inferior to the other two crosses from the 1991 year-class. Growth rate of 2N At × Bn hybrids is reported to be lower than that for 2N Atlantic salmon in both fresh and salt water (Refstie and Gjedrem, 1975; Refstie, 1983). No studies have been published on the growth performance of 3N At × Bn hybrids.

Some of the differences in relative growth rates observed in our experiments may be due to genotype by environment interactions. One such factor might be water temperature. In the early growth trials performed at WSU, the average temperature was 14.4°C, which is intermediate to the optimal rearing temperatures reported for Atlantic salmon (16–17°C) (Dwyer and Piper 1987; Jensen et al. 1989) and for brown trout (13°C) (Elliot 1975). The Stolt Sea Farms experiment was conducted at a much lower temperature (10.5°C) which may have favored growth of the 3N At × Bn hybrids. However, an effect of lower temperature favoring the hybrids is not clearly indicated in the two WSU late freshwater growth trials. In the 1990 year-class no differences in growth were observed between the At × At (2N), At × At (3N), and At × Bn (3N) crosses (average water temperature = 15°C). In the late trial with the 1991 year-class fish (average water temperature = 11.5°C), growth of the At × Bn (3N) was greater than that for the At × At (2N), but similar to that of the At × At (3N). Further testing will be needed to confirm and quantify the magnitude of any temperature effect. Culling of large and small fish prior to stocking, stocking density, light regime, and other factors that differed between the trials could also have led to variability in the relative growth rates.

In the WSU early (age 0+) freshwater growth trials, the At × Bn hybrids (2N and 3N) had consistently greater increases in coefficient of variation for weight than did the 2N and 3N Atlantic salmon. The At × Bn (3N) fish in the Stolt early growth trial also had a much greater coefficient of variation for final weight than the At × At (2N). In the WSU late (age 1+) freshwater growth trials, the coefficients of variation for weight increased or decreased only a small amount. Comparisons of variability in growth rate for salmonids and their hybrids have rarely been reported. Data from Refstie and Gjedrem (1975) and Refstie (1983) indicate no clear pattern of increased variability in growth rate for the 2N hybrids among Atlantic salmon, freshwater brown trout, anadromous brown trout, and Arctic char relative to the parental species.

A distinct group of "runts" were observed in the 1990 year-class late growth trial among the At × Bn (3N) fish. Of the third of the fish from this cross with the lowest initial stocking weights, 80% also had distinctly lower than average growth rates. No runts were observed among the At × At 2N and 3N fish. (Recall that the largest and smallest 20% of the fish from the 1990 year-class were culled at age 6.5 mo after initiation of feeding.) This effect was also observed in the 1991 year-class late growth trial, although it was less pronounced. The higher incidence of runts among the hybrids in these experiments is consistent with the significantly greater coefficients of variation for size that were observed for the hybrids in both WSU early growth trials and the Stolt Sea Farms trial. Uniformity is a desired character in a cultured fish stock. Additional grading would have to be performed during the presmolt period for commercial production of the triploid hybrid, and the percentage of fish culled would likely be higher than that for Atlantic salmon.

Commercial interest in production of At × Bn hybrids will require that they perform comparably to the currently cultured Atlantic salmon, and have a high assurance of sterility. Our experiments indicate that both the 2N and 3N At × Bn hybrids are as viable as Atlantic salmon of the same ploidy. The five freshwater growth trials performed with these fish indicated no consistent difference in growth rate, although the hybrids demonstrated greater variability in growth at age 0+. Continued study of the relative growth rates of the At × At (2N) and At × Bn (3N) crosses in saltwater net pens has been initiated, and fertility of the hybrids will be tested in backcrosses to the parental species.

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Survival to Fry and Seawater Tolerance of Diploid and Triploid Hybrids between Chinook (*Oncorhynchus tshawytscha*), Chum (*O. keta*), and Pink Salmon (*O. gorbuscha*)

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We investigated the viability and seawater tolerance of all possible combinations of triploid and diploid hybrids between chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), and pink salmon (*O. gorbuscha*) in two brood years. Triploidy (induced by heat shock shortly after fertilization) was determined by flow cytometry of blood taken from a sample of the fish. A factorial mating design was used to estimate the effects of cross, heat shock (ploidy), and their interaction. With the exception of the chinook (♀) × chum (♂) cross, all combinations produced offspring that survived to yolk absorption. Survival of heat-shocked groups was lower than that of diploid controls at the eyed stage and at hatching, but not different from diploids between hatching and yolk absorption. Induced triploidy did not increase the viability of interspecies hybrids. The level of triploid induction averaged across all heat-shocked crosses was 88% in one year and 95% in the other. Seawater tolerance (measured by 24-h seawater challenge survival) was higher in chinook hybrids with pink and chum salmon than in chinook controls. Seawater tolerance did not differ between diploid controls and heat-shocked triploid groups. Combinations that had seawater tolerance shortly after yolk absorption include chinook × pink, pink × chinook, and chum × chinook.

Nous avons étudié la viabilité et la tolérance à l'eau de mer de toutes les combinaisons possibles d'hybrides triploïdes entre saumons chinook (*Oncorhynchus tshawytscha*), kéta (*O. keta*) et rose (*O. gorbuscha*) de deux années de génération différentes. La triploïdie (induite par choc thermique peu après le fécondation) a été déterminé par cytométrie en flux à partir de sang prélevé chez un échantillon des poissons. Un plan d'accouplement factoriel a été utilisé pour estimer les effets du croisement, du choc thermique (ploïdie) et de leurs interactions. À l'exception du croisement chinook (♀) × kéta (♂), toutes les combinaisons ont produit une progéniture qui a survécu jusqu'à la résorption du sac vitellin. La survie des groupes traités par choc thermique a été plus faible que celle des témoins diploïdes au state oculé et au moment de l'éclosion, mais n'était pas différente de celle des diploïdes entre l'éclosion et la résorption du sac vitellin. La triploïdie induite n'a pas augmenté la viabilité des hybrides interspécifiques. Le taux moyen d'induction de la triploïdie pour l'ensemble des croisements traités par choc thermique a été de 88 % une année et de 95 % l'autre. La tolérance à l'eau de mer (mesurée par la survie après exposition de 24 h à de l'eau de mer) a été plus élevée chez les saumons chinook hybridés avec des saumons roses et kéta que ches les saumons chinook témoins. La tolérance à l'eau de mer n'a pas été différente entre les témoins diploïdes et les groupes triploïdes traités par choc thermique. Parmi les combinaisons viables qui présentaient une tolérance à l'eau de mer peu après la résorption du sac vitallin figuraient les croisements chinook × rose, rose × chinook et kéta × chinook.

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Interspecific salmonid hybrids may blend the desirable characteristics of both parents. The development of these fish for enhancement may offer a variety of advantages and options for culturists and fishery managers (e.g., Chevassus 1979; Ihssen et al. 1990). Modification of characteristics such as growth rate, timing of maturation, disease resistance, flesh

quality, and seawater tolerance may enhance culture potential and product value. Examples of the appearance of desirable characteristics in salmonid hybrids include disease resistance (Dorson and Chevassus 1985; Parsons et al. 1986; Dorson et al. 1991), earlier seawater tolerance (Novotny 1975; Seeb et al. 1993), and increased growth (Scheerer et al. 1987).

Earlier work (Novotny 1975; Utter et al. 1983; Seeb et al. 1988) identified crosses involving chum salmon (*Oncorhynchus keta*), pink salmon (*O. gorbuscha*), and chinook salmon (*O. tshawytscha*) as ones with potential for a variety of fish culture and enhancement applications. Chinook salmon are larger and more valuable than pink or chum salmon. Pink and chum salmon, however, tolerate seawater as fry, whereas chinook salmon generally require a freshwater rearing period (Weisbart 1967). Chum (♀) × chinook (♂) salmon hybrids (Seeb et al. 1993), chinook (♀) × pink (♂), and pink (♀) × chinook (♂) hybrids (Novotny 1975) demonstrated early seawater tolerance when compared with chinook controls. Early seawater tolerance is advantageous because the freshwater rearing of smolts is expensive, and is often a limiting factor in production. Also, in certain regions early transfer to seawater increases growth owing to favorable seawater temperatures.

Many intergeneric salmonid hybrids are almost totally sterile (Chevassus 1979), thus eliminating the energetic costs of reproduction and preventing introgression into wild salmon systems. However, some fertility may occur in hybrids within salmonid genera (Suzuki and Fukada 1973; Novotny 1975). Induced triploidy should reduce or, perhaps, eliminate the fertility of intrageneric hybrids because triploidy further impairs sexual maturation and reproduction (Lincoln and Scott 1984). Induced triploidy also improves the viability of some interspecific salmonid hybrids (Chevassus et al. 1983; Scheerer and Thorgaard 1983; Arai 1988; Blanc and Poisson 1988; Seeb et al. 1988; Gray et al. 1993).

The focus of our experiments is to evaluate the results of hybridization and ploidy manipulation for chinook, pink, and chum salmon in a production context. To do this we produced large numbers of fish to evaluate throughout their life cycle. The fish were and are being cultured in system designed for the captive rearing of salmon. Because production of sterile fish for culture is essential in some culture systems, we investigated triploid hybrids as well as diploid hybrids. Here we present results of the early culture that include the early survival of the interspecific diploid and triploid hybrids and the tolerance to seawater of their fry.

Materials and Methods

Experimental Design

Brood Year 1989 (BY89)

On 25 August 1989, we collected gametes from mature chinook, chum, and pink salmon to perform both triploid and diploid hybrid and control crosses for all possible pairs of species. Chinook salmon gametes were obtained from anadromous hatchery returns to the National Marine Fisheries Service (NMFS) Little Port Walter Field Station at Sashin Creek, on southern Baranof Island in southeastern Alaska. Chum and pink salmon gametes were taken from wild spawners in both Sashin Creek and nearby Lovers Cove Creek.

A total of 54 crosses were made using three replicates of a three by three factorial design for both diploid and triploid families. The gametes from each female were divided among the three male species and each aliquot of eggs was fertilized using the sperm of one male. This was repeated three times using different individuals from each species. The number of eggs per female varied among individuals and species. Sashin Creek water (9.7°C) was used to activate the gametes. Heat-shocked and control groups were created within each hybrid and single species cross. Ten minutes after fertilization a 20-min 26°C heat shock was applied to induce triploidy through retention of the second polar body (Chourrout 1980, Thorgaard et al. 1981, Utter et al. 1983; Seeb et al. 1988). Because of the scale of the project and the use of three different species, no attempt was made to optimize induction methodology. Control groups were activated with and incubated in ambient Sashin Creek water. Embryos were incubated in Heath Techna[1] vertical tray incubators, modified to create four isolated incubation compartments in each tray. Individual crosses were randomly assigned to positions in the incubation array. Flow rates were adjusted to approximately 19 L/min.

Brood Year 1990 (BY90)

On 17 August 1990 crosses were made using an experimental design similar to that of BY89 but with some modifications. The chinook (♀) × chum (♂) cross was not made, because chum gametes were difficult to obtain in 1990 and the cross had not been viable in our 1989 experiment or in Seeb et al. (1988). The chum (♀) × pink (♂) cross was not made in 1990 because of the limited availability of chum eggs. Two additional sets of factorial matings between pink and chinook salmon were made in a two by two array for diploid control and heat-shocked treatments. Ambient water temperature for fertilization was 14.5°C. Ten minutes after fertilization a 20-min, 28°C heat shock was applied to triploid experimental groups. The higher heat shock used to compensate for the higher ambient water temperature was suggested by J. Seeb (Alaska Department of Fish and Game, Anchorage , AK, personal communication).

Survival Data

We determined survival for each cross at the eyed embryo stage, at hatching, and again at yolk absorption. Survival for each time period was defined as the number alive at the end of the period divided by the number alive at the beginning of the period. Overall embryo survival was the number alive at yolk absorption divided by the initial number of unfertilized eggs in each incubation cell. We did not pick eggs after fertilization and heat shock to remove inviable eggs. Therefore, the mortality levels we have are maximum estimates and include mortality resulting from low gamete quality.

Ploidy Determination

Blood samples were collected from five fish of each heat-shocked group and from some control groups after yolk absorption as they were transferred from incubators to freshwater rearing units (ponded). Blood was collected by removing the caudal fin at its base and placing the fry into a 1.5-mL microfuge tube containing 0.5 mL of an anticoagulant solution (2% glucose, 0.8% trisodium citrate, and 0.4%

[1]Use of this product does not imply endorsement by the National Marine Fisheries Service.

TABLE 1. Average survival of brood year 1989 (1) from unfertilized eggs to the eyed embryo stage, (2) from the eyed stage to hatching, (3) from hatching to completion of yolk absorption, and overall survival from unfertilized egg to yolk absorption. Survivals reported were averaged over the families of each type of cross. The average percentage of triploidy was estimated at yolk sac absorption (Ys) and at about 1 yr (Yr). SWC= survival following a 24-h seawater challenge; P = presumed ploidy of the cross; R = number of replicates per cross; E = average number of unfertilized eggs per replicate.

					% Survival				% Triploid[b,c]		
Female	Male	P	R	E	1	2	3	Overall[d]	Ys	Yr	SWC
Chinook	Chinook	2	3	580	84	97	100	81	—	—	29
Chinook	Chinook	3	3	607	76	73	95	52	66	80	11
Chinook	Chum[a]	2	3	611	21	0	—	0	—	—	—
Chinook	Chum[a]	3	3	556	54	0	0	0	—	—	—
Chinook	Pink	2	3	539	81	92	99	74	—	—	67
Chinook	Pink	3	3	628	62	69	96	41	93	87	87
Chum	Chinook	2	3	555	78	90	86	60	—	—	58
Chum	Chinook	3	4	1638	49	87	95	39	80	90	73
Chum	Chum	2	3	380	90	100	99	88	—	—	77
Chum	Chum	3	3	370	61	86	96	50	92	93	60
Chum	Pink	2	3	392	87	93	93	76	—	—	100
Chum	Pink	3	3	470	56	81	95	42	90	100	84
Pink	Chinook	2	3	294	64	73	93	67	—	—	84
Pink	Chinook	3	4	711	74	92	99	67	95	90	95
Pink	Chum	2	3	234	96	99	100	95	—	—	98
Pink	Chum	3	3	301	89	93	96	79	87	80	100
Pink	Pink	2	3	255	97	99	100	96	—	—	100
Pink	Pink	3	3	336	90	89	99	79	93	100	100

[a]Only one of the chinook (♀) × chum (♂) crosses survived to hatching.
[b]A sample of 35 presumed diploids taken from the diploid groups were all diploid.
[c]On average, 86% of the heat-shocked fish were triploid at yolk absorption and 90% at 1 yr.
[d]Because the averages are based on data from individual families, the overall average may not be the product of survival at each developmental stanza.

sodium chloride). Samples were shipped on ice to the Department of Pathology, University of Washington, Seattle, WA, for analysis. Ploidy levels were assessed using flow cytometry (Thorgaard et al. 1982). Five additional fish from each BY89 cross were sampled in July 1990 and analyzed for ploidy.

Seawater Challenge

In mid-April, after approximately 90 d of freshwater rearing, 15 (BY89) or 25 (BY90) fry of each cross were taken from the freshwater rearing containers, placed in plastic containers, and exposed to full strength seawater (29–31 ppt) for 24 h. Survival of a 24-h exposure to seawater is an index of ability to withstand marine rearing (e.g., Schreck et al. 1985). We calculated survival from the number of live and dead fry in the containers at the end of the exposure period.

Statistical Analysis

We analyzed the data from the factorial design with an analysis of variance (ANOVA). The effects of cross (female species × male species combinations), and ploidy (heat shock or control) on embryo viability (three measurements) and survival after a 24-h seawater challenge were estimated with the model

$$Y_{ijk} = \mu + C_i + P_j + C \times P_{ij} + e_{ijk}$$

where Y is the dependent variable, percent survival, μ is the overall mean, C is cross (female species × male species) effect, P is ploidy using heat-shock as a surrogate for triploids, $C \times P$ is the interaction effect, and e is random error.

Survival variables were transformed (arcsine square root) before analysis to normalize the variance of binomial origin. If significant effects were detected with the ANOVA, Tukey's honest significant difference multiple pairwise comparison test was performed to test for pairwise differences. Overall significance for each variable was held at $\alpha = 0.05$. All statistical analyses were by SAS statistical procedures (SAS Institute Inc. 1982). The gametes of female chum 3 used in the BY89 matings were inviable; no crosses involving them were included in the analysis.

Results

We could generally distinguish hybrid fry from the pure species by their conformation and parr marking. Subsequently, we have been able to also distinguish them from their markings and growth performance (J.E. Joyce, R. Heintz, W.W. Smoker, and A.J. Charrett, Unpublished data)

Triploid Induction

Different heat shock regimes were applied in BY89 and BY90 because of the differences in ambient water temperature. In 1989, the ambient water temperature was 9.7°C, and treatment water was heated to 26°C. In 1990 the ambient water was 14.5°C, and treatment water was heated to 28°C. Although the proportion of triploids in BY89 heat-shock groups varied from 20 to 100%, the average was

TABLE 2. Average survival of brood year 1990 (1) from unfertilized eggs to the eyed embryo stage, (2) from the eyed stage to hatching, (3) from hatching to completion of yolk absorption, and overall survival from unfertilized egg to yolk absorption. Survivals reported were averaged over the families of each type of cross. The average percentage of triploidy was estimated at yolk sac absorption (Ys). SWC = survival following a 24-h seawater challenge; P = presumed ploidy of the cross; R = number of replicates per cross; E = average number of unfertilized eggs per replicate.

					% Survival				% Triploid[b,c]	
Female	Male	P	R	E	1	2	3	Overall[d]	Ys	SWC
Chinook	Chinook	2	5	782	90	99	99	89	—	88
Chinook	Chinook	3	5	901	71	46	90	33	95	80
Chinook	Pink	2	5	942	43	98	98	40	—	100
Chinook	Pink	3	5	978	25	63	96	15	100	96
Chum[a]	Chinook	2	5	144	75	81	81	47	—	87
Chum[a]	Chinook	3	5	313	11	38	98	4	100	90
Chum[a]	Chum	2	3	182	30	63	56	18	—	100
Chum[a]	Chum	3	3	334	6	12	0	0	100	—
Pink	Chinook	2	5	288	41	59	84	22	—	99
Pink	Chinook	3	5	292	20	75	87	12	95	99
Pink	Chum	2	3	233	28	66	66	26	—	100
Pink	Chum	3	3	226	15	57	63	13	100	100
Pink	Pink	2	5	282	46	99	100	55	—	100
Pink	Pink	3	5	334	19	66	94	16	85	100

[a]The quality of chum eggs was very low.
[b]A sample of 15 presumed diploids taken from the diploid groups were all diploid.
[c]On average, 95% of the heat-shocked fish were triploid at yolk absorption.
[d]Because the averages are based on data from individual families, the overall average may not be the product of survival at each developmental stanza.

86.8% at yolk absorption and 90.0% after 6 mo of freshwater rearing (Table 1). No spontaneous triploidy was observed in a sample of 35 diploid control fish. In BY90 heat-shock groups, the proportion of triploids ranged from 40 to 100% but averaged 95% at completion of yolk absorption (Table 2). Spontaneous triploidy was not observed in a sample of 15 control fish. Although a small number of individual crosses had low induction levels, the proportion of triploids observed in the samples of heat-shocked fish indicates a generally high level of induction. No significant effect of cross on ploidy induction levels was detected by the ANOVA in either brood year (Table 3).

Embryo Survival

Embryo survival measured at eyeing, from eyeing through hatching, and hatching through yolk absorption varied greatly among species, treatment groups, and brood years (Tables 1 and 2). Both cross and ploidy had significant effects on eyeing, hatching, and overall survival (Table 3). Only in the analysis of survival from hatching to ponding in BY90 was there no effect of ploidy or cross.

Average survival from unfertilized egg to completion of yolk absorption was higher in control than in heat-shocked groups ($P < 0.0001$; Table 3). Overall, mortality was higher in 1990 than in 1989 (Tables 1 and 2). Differences between heat-shocked groups and their controls were relatively high prior to hatching; but from hatching to completion of yolk absorption, survival differences between heat-shocked and control groups were relatively small (Tables 1 and 2).

All crosses produced some individuals that survived to completion of yolk absorption, except the chinook (♀) × chum (♂), which was viable until the eyed stage but failed to hatch (Table 1). For other hybrids, average survival from fertilization to the completion of yolk absorption tended to be lower than their pure species controls, but these differences were generally not significant in the pairwise comparisons ($P > 0.05$). An exception was the higher viability of chinook controls relative to chinook hybrids in BY90 ($P < 0.05$). Induced triploidy through heat shock did not improve viability in any of the interspecies hybrids during this stanza of life history.

Seawater Challenge

Early seawater tolerance accompanied chum and pink salmon contributions to hybrids. Pure chinook crosses had significantly lower survivals in pairwise comparisons with chinook hybrids in both brood years, although the absolute difference was smaller in BY90 (Tables 1–3). Any cross including either a pink or chum salmon as a parent experienced higher average survival in the seawater challenge. Control and heat-shocked groups did not differ in survival. Mean survival for control and heat-shocked crosses was similar in both BY89 and BY90 (Tables 1 and 2). The average seawater challenge survivals of BY90 crosses were higher than those of BY89.

Discussion

Average proportions of triploid induction were lower in 1989 crosses than in 1990 crosses; however, survivals were higher in 1989. Some of the lower survival is undoubtedly due to poor gamete quality in the chum salmon, which were collected near the end of the run in 1990 and in unusually high stream temperatures. The higher temperature shocks may also have increased mortality but at the same time appear to have increased the efficiency of triploidization. Because we did not conduct experiments to determine the optimum postfertilization time or the ideal temperature and

duration of shock, we cannot separate the effects of our heat-shock regime from other sources of interannual variation, such as egg quality and subsequent incubation conditions. Triploids did result from our heat-shock regimes in eight of the nine different crosses we made, and the other cross was inviable (chinook (♀) × chum (♂)).

Although the number of fish sampled per heat-shocked cross was relatively low (10 each for BY89 and 5 each for BY90), the total sample for the entire experiment was large (250 for BY89 and 100 for BY90). The high proportion of triploidization (86% for BY89 and 95% for BY90 at yolk absorption) observed in the sample indicates that the heat shock treatments we used were mostly successful in producing triploids. Moreover, the high rate of spontaneous triploidy reported in a similar study involving pink salmon (Utter et al. 1983) was not observed in our study. No triploids were observed in our sample of 50 fish from diploid control groups.

The variation among families within a species in the yield of polar body retention has also been observed in Pacific salmon gynogens (Levanduski et al. 1990; Smoker et al. 1995) and in some salmon hybrids (Seeb et al. 1988). Such variation is undoubtedly influenced by gamete quality, other experimental variables, and genetic variation.

Survival of the diploid and triploid crosses was examined in three stanzas: (1) from unfertilized egg to eyed egg, (2) from eyed egg to hatching, and (3) from hatching until completion of yolk absorption when the fry were transferred from the vertical tray incubator to larger freshwater containers. Survivals of fish from the BY89 crosses were generally high, as was previously reported (Utter et al. 1983; Novotny 1975), exceeding 75% for pink and chum diploid controls. Patterns of survival for BY89 and BY90 embryos during each development stanza indicate that the heat-shock treatment increases mortality prior to hatching.

In the first stanza, from unfertilized egg to eyed egg, families from both brood years experienced greater mortality in the heat-shocked crosses than in the diploid crosses from the same parents. Mortalities from eyed egg to hatching followed the same pattern. In contrast, there was no significant difference in the survival of diploid and heat-shocked crosses between hatching and completion of yolk absorption. The early mortality of the presumed triploid groups appears to occur primarily before hatching. In a fish-culture context it makes no difference whether the mortality results from impairment of the development process by thermal shock to cytoplasmic elements or from triploidization or aneuploidy because the mortality occurs before there has been a large investment in rearing space and conditions or food. Of course, optimization of heat-shock conditions may increase the early survival and rate of triploidization.

When triploidy is induced by temperature shock, crosses involving a single species experience lower survivals to hatching than do control diploid groups (Happe et al. 1988), but induced triploidy increases the viability of certain salmonid hybrids (Seeb et al. 1988; Gray et al. 1993). In our study, heat-shocked treatment groups, including hybrids, generally experienced lower survival than diploid controls. An exception is the 1989 chinook (♀) × chum (♂) heat-shocked cross, which had higher survival than diploid controls until the eyed stage. Both control and heat-shocked chinook (♀) × chum (♂) subsequently died before hatching. In 1990, there was not a single case of a heat-shock group experiencing higher survival from unfertilized egg to yolk absorption.

TABLE 3. Summary of probabilities of main effects tested by analysis of variance for the survivals of transformed survivals of progeny from brood years 1989 (BY89) and 1990 (BY90). Survival was modelled as the sum of the effects of cross (parent species), ploidy (heat shocked or not), and the interaction of cross and ploidy. ns = not significant.

		Probability	
Variable	Effect	BY89	BY90
Survival to eyed stage	Cross	0.0004	0.0001
	Ploidy	0.0625	0.0005
	C × P	0.0652[a]	ns
Survival from eyed to hatching	Cross	0.0001[b]	0.1199
	Ploidy	0.0001[a]	0.0001[a]
	C × P	0.0090	0.0114
Survival from hatching to completion of yolk absorption	Cross	0.0205	ns
	Ploidy	ns	ns
	C × P	0.0254	ns
Survival from unfertilized egg to completion of yolk absorption	Cross	0.0001	0.0008
	Ploidy	0.0001	0.0001
	C × P	ns	ns
Survival after 24 h seawater challenge	Cross	0.0001[c]	0.0001[c]
	Ploidy	0.5545	0.4033
	C × P	0.7975	0.6938

[a]The 3N crosses had lower survivals than 2N crosses.
[b]Chinook (♀) by chum (♂) cross failed in BY89. The cross was not included in BY90.
[c]Pure chinook crosses performed poorly compared with crosses including pink or chum parents.

The survival we observed for heat-shocked chum (♀) × chinook (♂) was lower than that of the diploid control crosses of this type in BY89 and lower (39%) than that previously reported (76%) by Seeb et al. (1988). The very low survival of the BY90 crosses probably reflects poor gamete quality. Significant individual variability observed in these studies may explain the disparity in results, especially because the number of individual females spawned was relatively small in both cases (two for Seeb et al. 1988, and six in this study).

Seawater tolerance was higher in chinook hybrids with pink and chum salmon than in pure chinook controls. This difference was more dramatic in 1989, when survivals were generally lower for all crosses. A greater seawater tolerance was observed for the chum (♀) × chinook (♂) cross relative to pure chinook crosses (Seeb et al. 1993), and seawater adaptability was also high in pink (♀) × chinook (♂) and chinook (♀) × pink (♂) hybrids (Novotny 1975). The ability to tolerate seawater as fry makes these combinations attractive for marine culture and fisheries enhancement. The survival of heat-shocked groups was not different from that of the diploid controls, in contrast to other work in which triploid coho salmon experienced higher mortalities than diploid controls under certain conditions during seawater adaption (Johnson et al. 1986). The pink (♀) × chinook (♂), chinook (♀) × pink (♂), and chum (♀) × chinook (♂) are the most promising of the crosses we examined. These viable hybrids survived at high rates in both heat-shocked and control groups, and exhibited superior seawater tolerances when

compared with chinook controls. It will be important to determine the extent of their development of secondary sex characteristics and fertility in evaluating their utility in fish culture. Studies of these crosses, their suitability for culture, and their sterility continue. Fry from surviving families were transferred after yolk absorption to individual freshwater rearing containers (Heintz and Joyce 1992) and subsequently to seawater net pens. Present investigations include measurement of their growth and survival rates in fresh and seawater, flesh quality, maturation schedules, and gamete development.

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Triploid Coho Salmon Outperform Diploid and Triploid Hybrids Between Coho Salmon and Chinook Salmon During Their First Year

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Sterile hybrid and triploid fish may provide hatchery programs with a tool to reduce the risk of genetic contamination of wild stocks, provided these fish have acceptable performance characteristics. We examined growth, survival, and deformities in diploid and triploid families of coho salmon (*Oncorhynchus kisutch*) and hybrids between coho salmon females × chinook salmon (*O. tshawytscha*) males. Data were collected from the half-sibling families through day 387. A reparameterized Gompertz growth model showed that conspecific coho salmon grew faster than hybrids, regardless of ploidy. No difference in growth rates was found between diploids and triploids. Abnormalities were significantly associated with the male parent but not with ploidy component, in contrast to previous observations of reduced deformity occurrence in triploid hybrids. Food conversion was better for conspecifics than hybrids during initial feeding, but not different during the second half of the experiment. No ploidy or cross × ploidy interaction effects on food conversion efficiency were observed. Finally, conspecifics had better posthatching survival than hybrids, regardless of ploidy. Of the treatments studied, conspecific triploid coho salmon may be the most viable alternative for sterile fish production: they performed as well as the conspecific diploids and better than either diploid or triploid hybrids.

Des poissons hybrides et des poissons triploïdes stériles pourraient être utilisés dans les programmes d'élevage pour réduire le risque de contamination génétique des stocks sauvages s'ils présentaient des caractéristiques de rendement acceptables. Les auteurs ont examiné les taux de croissance, de survie et de difformité chez des familles de saumons cohos (*Oncorhynchus kisutch*) diploïdes et triploïdes et des hybrides obtenus de croisements de saumons cohos femelles et de saumons quinnats (*O. tshawytscha*) mâles. Ils ont recueilli des données sur les familles à demi-apparentées pendant 387 jours. Une courbe de croissance reparamétrée de Gompertz a révélé que le taux de croissance des cohos congénères était plus élevé que chez les hybrides, indépendamment du degré de ploïdie; par contre, il n'y avait pas de différence de ce taux entre les saumons diploïdes et triploïdes. Les anomalies étaient liées de façon significative au parent mâle, mais non à la ploïdie; ceci va à l'encontre d'observations antérieures selon lesquelles les hybrides triploïdes montrent un taux moindre de difformité. Le taux de conversion des aliments était plus élevé chez les congénères que chez les hybrides à la première prise d'aliments, mais n'était pas différent pendant la deuxième moitié de l'expérience. Les auteurs n'ont pas observé d'incidence du degré de ploïdie ou de l'interaction croisement et ploïdie sur l'efficacité de conversion des aliments. En outre, les congénères présentaient un meilleur taux de survie après l'éclosion que les hybrides, indépendamment du degré de ploïdie. Des traitements étudiés, celui des saumons cohos congénères triploïdes semble constituer la meilleure solution rentable pour la production de poissons stériles : leur rendement est semblable à celui de leurs congénères diploïdes et meilleur que celui des hybrides diploïdes ou triploïdes.

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Wild stocks of Pacific salmon (*Oncorhynchus* spp.) support important commercial and sport fisheries around the North Pacific Ocean. Over the past decades these stocks have been supplemented with huge hatchery releases from Japan, United States, Russia, and Canada. Recent evidence from Europe and North America indicates that hatchery and net-pen cultured salmonids can have detrimental genetic effects on wild stocks (for reviews see Windsor and Huchinson 1990; Allendorf 1991; Hindar et al. 1991) and has prompted concern over introgression of hatchery genes into wild stocks in Pacific salmon (Krueger and May 1991; Waples 1991; Hilborn 1992).

Steps taken to alleviate these concerns with Pacific salmon include attempts at the exclusive use of local stocks (e.g., Krueger et al. 1981; Davis et al. 1985), releasing fish at times and locations that minimize straying, and, in Alaska, outlawing net-pen culture. However, these steps are not completely effective at eliminating hatchery stock genetic introgression into wild stocks. There is mounting evidence that (1) hatchery runs genetically diverge from their wild-stock

TABLE 1. Number of fish in each experimental cell at the beginning of evaluation periods 1 (days 87–227) and 2 (days 227–387). Diploids and triploids are designated as 2N and 3N, respectively.

Family no.	Evaluation period	Conspecifics		Hybrids	
		2N	3N	2N	3N
1	1	50	16	49	24
	2	31	15	7	14
2	1	51	6	46	46
	2	—[a]	—	—	—
3	1	47	47	47	34
	2	33	11	30	19
4	1	44	51	43	48
	2	23	41	21	21

[a]Family No. 2 was removed from the experiment after the first period due to small sample size in the conspecific triploid cell.

founding populations (Allendorf and Phelps 1980; Ferguson et al. 1991), (2) some hatchery practices induce straying (Larsson 1984; Eriksson and Eriksson 1991; Hindar et al. 1991; Hindar 1991), and (3) escaped net-pen fish have negatively impacted wild stocks (Gausen and Moen 1991; Mork 1991) and do not necessarily heed international borders (Wing et al. 1992). Additionally, management agencies still conduct extensive planting of fish for sport angling, often using a single stock of fish for stocking over a broad geographical area.

Introgression of hatchery-stock genes into wild stocks may be prevented by requiring the use of sterile fish in some aquaculture and stocking programs. Until a better understanding of the migratory behavior of sterile individuals of this anadromous genus is available, nonexperimental applications may best be limited to captive culture or stocking into landlocked lakes.

Two standard methods to produce sterile fish are triploidization and interspecific hybridization (Ihssen et al. 1990). Both have individually produced sterility in many species including salmonid species (Allen and Stanley 1978; Thorgaard 1983; Lincoln and Scott 1984; Allen et al. 1986; Benfey et al. 1987). But, because diploid hybrids are not always sterile, producing triploid hybrids should provide a better guarantee of sterility.

In salmonids, triploidization has also yielded increased survival in interspecific hybrids over their diploid counterparts (Chevassus et al. 1983; Scheerer and Thorgaard 1983; Arai 1986; Scheerer et al. 1987; Seeb et al. 1988; Yamano et al. 1988). Triploid hybrids have sometimes expressed increased growth (Stanley et al. 1984; Benfey et al. 1989), improved disease resistance (Dorsen and Chevassus 1984; Parsons et al. 1986; Dorsen et al. 1991) and earlier saltwater tolerance (Seeb et al. 1993) compared with one or both of their parental species.

Coho salmon (*O. kisutch*) and chinook salmon (*O. tshawytscha*) are economically important species of Pacific salmon. They are commercially farmed in Pacific Rim countries and often stocked in landlocked lakes in Alaska. Consequently, the study of sterile fish could have important implications for coho salmon and chinook salmon management.

Conflicting results have been observed with triploid coho salmon. Utter et al. (1983) found reduced growth in triploid coho salmon compared to diploids, but Johnson et al. (1986) found no difference between these two groups. Although both diploid and triploid coho female × chinook male hybrids have shown encouraging early survival and are prime candidates for sterile fish production (Seeb et al. 1988), no postlarval performance studies have been done.

In this study we examine the growth, survival, food conversion efficiency, and rates of deformity in diploid and triploid coho salmon and hybrids between coho salmon females and chinook salmon males. The assessment of such parameters is essential to determine the potential of sterile-fish treatments for either aquaculture or stocking programs. Our findings indicate that, of the treatments studied, conspecific triploid coho salmon may be the most viable alternative; they performed equal to the conspecific diploids and better than either diploid or triploid hybrids.

Methods

Matings

Chinook salmon milt was collected from Deception Creek, Alaska, in July 1991. Milt was cryopreserved using the methods of Wheeler and Thorgaard (1991). Coho salmon eggs and milt were collected from Nancy Lake, Alaska, in October 1991 and transported green to the Broodstock Development Center, Fort Richardson, Alaska.

Eggs from each of 10 females were subdivided in two lots. For each female, one lot was fertilized with a single chinook salmon male and the other with a single coho salmon male. Different males were used with each female. Each lot was further subdivided into two sublots. One chinook salmon fertilized sublot and one coho salmon fertilized sublot were heat shocked 20 min postfertilization for 20 min at 26°C (ambient water temperature was 8°C) to induce second polar body retention to produce triploids, a modification of Chourrout (1980). The other two sublots were not heat shocked. Each sublot is one of four treatments per family and is defined as a family treatment.

Embryos in each family treatment were incubated separately to hatching in divided incubator trays at 6.7°C. The four families with highest survival in the family treatment of lowest survival were placed in 16 tanks (one tank for each family treatment) for growth trials. The intent of this choice of families was to obtain the largest numbers of individuals per cell of the experiment for cells with low initial numbers.

Fish Rearing

Sixteen family-treatments (four treatments per family × four families) with a mean of 41 fish per treatment were reared in separate 11-L circular tanks until 227 d postfertilization at 12.9°C (±2°C) (Table 1). At this time, all four treatments from one family were removed from the growth trials due to a low initial number and a high mortality in one of the treatments during this first growth period. The 3 remaining families (12 family treatments) with a mean of 21 fish per family treatment were reared in 70-L circular tanks to day 387 at 13.2°C (±0.1°C) (Table 1). The reduction in the number of fish per family treatment during the second evaluation period was due to mortality during the first growth period.

Fish were hand fed 10 times daily with BioDiet (Bio Products, 1935 N.W. Warrenton Drive, Warrenton, OR 97146,

TABLE 2. Parameter estimates for Schnute (1981) reparameterized Gompertz growth model.

Parameter[a]	Variable[b]		Estimate
w_0			0.326 6
z			0.007 396
k	Conspecifics	2N	0.007 105
		3N	0.007 154
	Hybrids	2N	0.004 697
		3N	0.004 632
w	Conspecifics	2N	50.42
		3N	50.54
	Hybrids	2N	37.13
		3N	45.29

[a]Parameters: w_0 = weight at hatch (held constant); k = relative growth of relative growth rate; w = weight on day 387; z = autocorrelation

[b]Variables: 2N = diploids; 3N = triploids

USA) starter diet until mean fish weight per tank was 1.05 g (129 days postfertilization). Fish were then hand fed to satiation five or six times daily with BioDiet Dry 1000 and the weight of food fed per tank was calculated daily through day 387 postfertilization.

Fish were counted and individually weighed every 2 wk from hatching through day 227 postfertilization and approximately every two months thereafter. MS-222 anesthetized fish were weighed unblotted to the nearest 0.01 g on a Mettler PC4400 balance. Gross abnormalities, consisting of vertically compressed caudal peduncles, were recorded 183 d postfertilization to determine whether hybridization or triploidization affects deformity rates.

Ploidy Analysis and Electrophoresis

We confirmed ploidy because thermal shocks have yielded varying degrees of triploidy in coho salmon (Parsons et al. 1986; Seeb et al. 1988) and because interspecific crosses have resulted in haploids and triploids (Chevassus 1983). At the termination of the experiment, blood from all 128 putative triploids, 8 putative conspecific diploids, and 35 putative hybrid diploids (some from each hybrid family) was taken and frozen in a nuclear isolation medium (4,6-diamidino-2-phenylindole; NIMS–DAPI) solution (Thornthwaite et al. 1980) with 10% dimethyl sulfoxide. Blood cells were analyzed for ploidy using flow cytometry (Thorgaard et al. 1982).

We confirmed biparental inheritance because interspecific matings have sometimes resulted in androgens or gynogens (Chevassus 1983; Johnson and Wright 1986). Muscle, heart, liver and eye samples were taken from parents during gamete collection and from all progeny at the end of the experiment. Samples were immediately placed on dry ice, transported to the laboratory, and stored at –80°C until analysis. Allozyme electrophoresis was performed following the methods of May et al. (1979) and the nomenclature of Shaklee et al. (1990). Eight diagnostic loci identified from analysis of parental tissues were used to document biparental inheritance in putative hybrids (Table 2).

Data Analysis

Growth and mortality analyses were performed on cell means, where a cell represented a family treatment for a sampling period. Use of cell means rather than individual fish measurements avoided pseudoreplication (Hurlbert 1984) due to tank effects. The alpha level was 0.05 for all tests.

TABLE 3. Tests between hybrids and conspecifics (H–C), hybrid diploids and hybrid triploids (H2–H3), and cross × ploidy interaction (CR × P) for k (relative rate of relative growth rate) and w (weight at time T) using the Schnute (1981) reparameterization of the Gompertz growth model. Asterisks indicate significant two-tailed t-tests (df = 158).

Parameter	Time (T)	Test	t	p
k		H–C	−2.79	0.005 88*
		H2–H3	0.0543	0.957
		CR × P	0.0656	0.948
w	387	H–C	−1.54	0.125
		H2–H3	−1.10	0.271
		CR × P	−0.691	0.491
	339	H–C	−2.51	0.013 0*
		H2–H3	−1.04	0.302
	297	H–C	−3.02	0.002 91*
		H2–H3	−0.840	0.402
	227	H–C	−3.22	0.001 54*
		H2–H3	−0.536	0.593

Statistical analyses were performed using the S-PLUS® statistical package. To use weight data from all the sampling dates (Hopkins 1992), the following Schnute (1981) reparameterization of the Gompertz growth model was fitted to the data using least squares and a multiplicative and autocorrelated error structure:

$$W(t) = w_0 \exp\left(\frac{1 - e^{-k(t-T_0)}}{1 - e^{-k(T-T_0)}} \ln\left(\frac{w}{w_0}\right) + \epsilon_t\right)$$

where $W(t)$ = weight on day t, $87 \le t \le 387$, w_0 = starting weight (day 87 postfertilization), w = weight on day T postfertilization, k = constant relative rate of relative growth rate, T_0 = starting day, $T_0 = 87$, T = day postfertilization, and ϵ_t = autocorrelated error at time t.

Because the weight measurements were made from the same fish over time (averages of each tank), the residuals were correlated. Therefore, autoregressive errors were added to the model (Bates and Watts 1988):

$$\epsilon_{t_i} = \gamma_{t_i} + \phi\,(t_i, t_{i-1})\epsilon_{t_{i-1}},$$

distribution with mean 0 and variance σ^2. Time was indexed by i because time intervals between measurements were not constant.

Because ϕ diminished as the time between intervals increased, an inverse exponential function was used to parameterize ϕ:

$$\phi(t_i, t_{i-1}) = \begin{cases} e^{-z(t_i - t_{i-1})} & i > 1 \\ 0 & i = 1 \end{cases}$$

thereby adding another parameter (z) to the estimate. The multiplicative error structure maintained heterogeneous variances over time.

We tested the effect of cross (hybrids versus conspecifics) and ploidy (diploids versus triploids) and cross × ploidy interaction on k and w by adding linear components for these two parameters to the model as is done for the general linear model. The asymptotic variances supplied by the nonlinear least squares function of the S-PLUS® statistical package

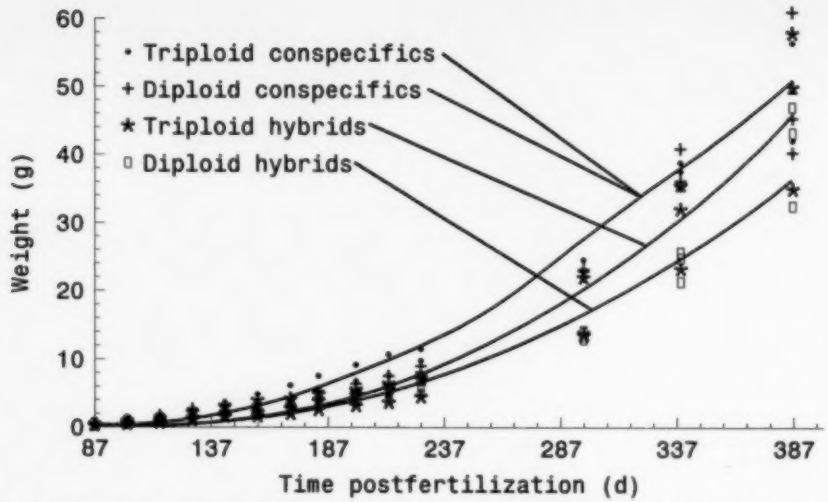

FIG. 1. Weights of coho salmon conspecific diploids and triploids and coho salmon female × chinook salmon male hybrid diploids and triploids for each sampling period for the three half-sibling families that were represented throughout the experiment. Predicted curves from the Schnute (1981) reparameterized exponential growth model are superimposed on the data.

were used to test contrasts of interest. Testing the differences in weights on any day was accomplished by setting T to the day of interest. The only estimates that changed during this procedure were the ones associated with w.

Only data from the three families that were represented throughout the experiment were included in this analysis. One estimate for starting weight was used for all treatments because the same females were used for each treatment, resulting in expected similar yolk-sack fry weights prior to feeding.

During the experiment the growth rate was increasing and had not reached its maximum (the inflection point of the growth function) after which the growth rate decreases. Therefore, the data could be viewed as incomplete for fitting a sigmoid growth curve that includes growth inflection and asymptotic size. However, we did not intend to make any inferences about growth beyond the scope of our data, and the Schnute reparameterization provided a means to statistically test differences in weights on any sampling date.

We also used an analysis of variance to test whether each effect contributed significantly to the model. This analysis was done by fitting the full model and then testing the increase in the residual sum of squares due to dropping each effect using an F-statistic (Neter et al. 1990).

A binomial generalized linear model using a logit link function (McCullagh and Nelder 1989) was used to test the effects of female, cross, ploidy, and cross × ploidy interaction on survival. Survival rates from day 87 (hatching) to day 387 postfertilization were used for this analysis.

Two-way ANOVA was used to test effects of cross, ploidy, and cross × ploidy interaction on food conversion for two evaluation periods. The first period, between days 129 and 227 postfertilization, includes data collected from the time food weight was measured and included the time fish were held in 11-L tanks. The second period, between days 227 and 387 postfertilization, included data collected through the end of the experiment while fish were held in 70-L tanks. The test was based on the mean of the food conversion efficiencies for all sample dates within the two evaluation periods. The food conversion efficiency, C, for each evaluation period was calculated as

$$C = \left\{\frac{F}{(N_i + N_f)/2}\right\}\left\{\frac{1}{W_i - W_f}\right\}$$

where F = food fed over period (g), N_i = number of fish at the beginning of the period, N_f = number of fish at the end of the period, W_i = mean weight of fish at the beginning of the period (g), and W_f = mean weight of fish at the end of the period (g).

This formula approximately accounted for mortality occurring during the evaluation period if the times fish died were random and independent of their size. These assumptions were supported by casual observations.

Results

The Gompertz growth model as reparameterized by Schnute (1981) showed hybrids and conspecifics had different k (constant relative rate of relative growth rates) but not different w on day 387 (Table 3). However, weight differences between hybrids and conspecifics were found on the first three of the last four sampling dates (Table 3) with conspecifics consistently outweighing hybrids (Fig. 1).

Diploids and triploids had almost identical parameter estimates within conspecifics but not within hybrids (Table 2). Even within hybrids, however, no differences between diploids and triploids for k or final weight were found (Table 3). In addition, no weight differences between hybrid diploids and triploids were found on the first three of the last four sampling dates (Table 3).

The analysis of variance produced the same conclusions verifying that the asymptotic variances produced by the nonlinear least squares function were good approximations of the true variances. Plot of residuals at time t_i versus residuals at time t_{i-1} showed a shotgun pattern indicating

that autocorrelation was eliminated. Normal quantile–quantile plot showed the data were lined up on the $y = x$ line indicating a normal distribution. Plot of residuals by time showed a slight, but insignificant, wave around the $y = 0$ line indicating homogeneity of variance.

Conspecific survival (62%) was higher than hybrid survival (43%) from hatching (day 87 postfertilization) through day 387 ($z = 4.98$; df = 10; $p = 0.000$). The same generalized binomial model showed no difference in survival rates between diploids and triploids ($z = 1.44$; df = 10; $p = 0.149$) and no interaction between ploidy and species ($z = 1.63$; df = 9; $p = 0.104$).

Food conversion efficiency was better for the conspecifics ($C = 1.54$) than for the hybrids ($C = 2.22$; $p = 0.010$) during the first evaluation period (days 129–227 postfertilization). However, no difference was detected between diploids and triploids ($p = 0.080$) nor interaction detected between cross and ploidy ($p = 0.890$) during this period. During the second evaluation period, food conversion efficiency was almost identical across all groups ($C = 1.15$, range 1.14–1.16; $p > 0.908$).

Deformities observed in this study appeared to be sire related rather than cross or ploidy related. Nighty-eight percent of the deformities appeared in the two families sired by chinook salmon male No. 5. One family had deformity rates of 66.7% ($N = 24$) for diploids and 63.9% ($N = 36$) for triploids; the other family had 15.0% ($N = 27$) for diploids and 21.4% ($N = 28$) for triploids. Within these families, no difference in abnormality rates was found between diploids and triploids. Half-siblings of these families sired by coho salmon had no deformities. No deformities appeared in families sired by either of the other two chinook salmon. The only other deformed fish observed was in an unrelated diploid conspecific family.

Ploidy and Biparental Inheritance

The triploidy rate in the heat-shocked families averaged 96.4%. Two families were 100% ($N = 44$) triploid, and the other two families were 96.3% ($N = 27$) and 94.5% ($N = 55$) triploid. All putative diploids screened, both hybrid and conspecific, were confirmed to be diploid.

All putative hybrids showed biparental inheritance for the diagnostic loci *sAH*, mAH-1*, mAH-4*, GPI-A*, MPI*, PEP-B1*, PEPD*, and PGM-2**. Of these loci, *sAH*, GPI-A*, and MPI** had the best resolution (separation, staining activity, and staining sharpness) for differentiating juvenile hybrids from conspecifics. *ALAT*, FH*, and sIDHP-2** did not provide sufficient resolution to differentiate hybrids from conspecifics.

Discussion

These data indicate that coho salmon triploids had the best growth and survival of the putative sterile fish tested, and performance of diploid and triploid coho salmon was similar during their first year. Importantly, however, this study terminated after 1 yr, well before either coho salmon or chinook salmon mature. Sterile fish may continue somatic growth as fertile fish reach sexual maturity and start shunting energy toward gamete production (Wolters et al. 1982; Lincoln and Scott 1984; Thorgaard 1986; Ihssen et al. 1990). Therefore, as long as the sterile fish are growing approximately as well as their fertile counterparts at the early life stages, they are good candidates for further growth trials as their fertile counterparts reach sexual maturity. One indication that hybrids may catch up in weight with conspecifics is the convergence in weight between triploid hybrids and conspecifics during the last sampling dates (Fig. 1) and the lack of a weight difference between hybrids and conspecifics on the last date (Table 3).

Our results showing no difference in the growth rates between diploids and triploids are in conflict with Utter et al. (1983) who found better growth in diploids than triploids. This discrepancy may have been caused by either a family (female) effect (they studied only one family) or competition between diploids and triploids which they raised in the same tank (ours were raised in separate tanks). Our results are in agreement with Johnson et al. (1986) who found no difference in growth rates between diploid and triploid coho salmon.

Although food conversion efficiency was better for the conspecifics than the hybrids during the first period of evaluation, food conversion efficiency was practically identical among all four treatments during the second period of evaluation. Because fish in later life stages consume more food, the results from the second half of this experiment are more important than the initial food conversion efficiencies. We did not observe better food conversion efficiency in the triploids, as Wolters et al. (1982) and Wolters and Lilyestrom (1991) have observed in catfish.

Our deformity data provide a compelling argument for conducting these kinds of experiments with either controlled family effects or large numbers of families in a pooled experimental design so that variation due to one individual does not lead to incorrect conclusions. When our data was initially analyzed, excluding the male effect, our data showed hybrids had higher ($p = 0.000$) deformity rates (24.6%; $N = 195$) than conspecifics (0.5%; $N = 211$). However, when we looked at deformity rates between families within crosses, we found that families sired by one chinook accounted for almost all the deformities (98%), whereas the rest of the families sired by other chinook had no deformities. Therefore, when applying statistics to data sets like this, family effects must be incorporated in the statistical model.

Our success in retaining second polar bodies in heat-shocked eggs (96.4%, $N = 126$) was better than that reported by Parsons et al. (1986) and Seeb et al. (1988) (38%, $N = 8$; 54%, $N = 64$, respectively). Although both earlier studies used higher temperatures, they initiated shock 10 min earlier than in this study. Parsons et al. (1986) used a shock of 20-min duration, as we did, whereas Seeb et al. (1988) used a 10-min duration. We observed similar low triploidy rates with the same coho salmon strain used in this study with a shock initiation time of 10 min postfertilization. The hypothesis that later shock initiation is important in achieving high triploidy in coho salmon contrasts with work done by Solar et al. (1984) on rainbow trout who recommended early shock. Also, Levanduski et al. (1990) found that the time of shock initiation did not significantly affect triploidy in chinook salmon. In coho salmon, repair of the microtubule spindle after shock application may be possible if the shock is performed too early.

As a consequence of functional sterility, hybridization and triploidization may also reduce the levels of precocity in males observed in both coho salmon (Silverstein and

Hershberger 1992) and chinook salmon (Hard et al. 1985; Mullan et al. 1992). In Alaska, for example, 5–10% hatchery production losses of males are attributed to precocity in chinook salmon.

In conclusion, triploid coho appear to have comparable growth, survival, deformity rates, and food conversion efficiency to diploid coho, making them the most promising candidates of the fish investigated in this study for sterile fish production. However, both diploid and triploid hybrids still warrant further study to investigate longer term growth and survival as diploid conspecifics reach sexual maturity.

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A Preliminary Ploidy Analysis of Diploid and Triploid Salmonids

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The findings indicate that mosaicism does occur in triploids and especially in triploid hybrids. Immature diploid and triploid salmon were obtained from the University of Alaska – National Marine Fisheries Service. These were fixed in 10% buffered formalin and blocks taken from various tissues including kidney, liver, spleen, and gonads. The paraffin blocks were sectioned at 4 μm and stained with hematoxylin and eosin for histologic evaluation and to quantitate nuclear DNA. The slides for DNA evaluation were stained using the Cell Analysis Systems, Inc. (CAS, Elmhurst, IL) kit following Feulgen hydrolysis. The Cell Analysis Systems, Inc. (Model 200) computer-assisted image analyzer was used for DNA quantitation. An attempt was made to evaluate the frequency of mosaicism, ploidy variation in somatic and gonadal tissues of triploids and hybrids as compared with normal diploids. Hepatic ploidy variation, normal in adult animals, was not present in these specimens. However, in gonads there was evidence of increased mosaicism among the triploids. Because of mosaicism in gonadal tissue, sterility of triploids cannot be presumed with certainty at this stage. Further studies are planned to examine this and other questions raised by these results.

Les résultats de la présente étude révèlent l'existence d'un mosaïcisme chez les saumons triploïdes, en particulier les saumons hybrides triploïdes. Les auteurs ont obtenu des saumons diploïdes et triploïdes immatures du National Marine Fisheries Service et de l'Université de l'Alaska, et les ont conservés dans du formol tamponné à 10 % avant de prélever des échantillons de divers tissus, notamment du rein, du foie, de la rate et des gonades, pour les inclure dans de la paraffine. Les coupes de 4 μm ont été colorées à l'hématoxyline et à l'éosine pour examen histologique et détermination de la quantité d'ADN dans le noyau. Après hydrolyse par réaction de Feulgen, les auteurs ont utilisé une trousse de Cell Analysis Systems (CAS, Elmhurst, IL) pour colorer les préparations microscopiques, et un analyseur informatique d'images de la même compagnie (modèle 200) pour déterminer la quantité d'ADN. Ils ont tenté d'évaluer la fréquence du mosaïcisme, soit la variation de la ploïdie dans les cellules somatiques et germinales des saumons triploïdes et hybrides par rapport aux saumons diploïdes normaux. Ils n'ont pas décelé de variation de la ploïdie dans les cellules hépatiques des saumons expérimentaux, variation qui est normale chez les saumons adultes; par contre, ils ont noté un mosaïcisme accru dans les cellules germinales des triploïdes, ce qui ne leur a pas permis d'établir avec certitude si les saumons triploïdes étaient stériles. Les auteurs prévoient mener des études plus poussées sur le sujet et d'autres questions soulevées par les résultats de la présente étude.

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The imperatives of the present aquaculture program in the United States have resulted in several programs of ploidy manipulation and hybridization among salmonids and other cultured fish. Measurements of the success of these programs, which includes survival rate, growth and development, and fertility, need to be correlated with the ploidy status of the progeny. Furthermore, because complex genetic and cytogenetic mechanisms may be involved in the development of triploids and hybrids, analysis of multiple somatic tissues, as well as the sex organs, are required. Previous studies in fish and other vertebrates have shown that mosaicism and genomic duplication in first meiosis may occur and may lead to unexpected phenotypic and fertility results (Murayama et al. 1986; Cherfas and Tzoy 1984).

This investigation concerns the aquaculture potential of diploid and triploid salmonid hybrids, involving the chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), and pink salmon (*O. gorbusha*). It is hoped that these varieties can be utilized

TABLE 1. List of the analyzed fish.

Label[a]	Fork length (cm)	Body weight (g)	Ploidy[b]
P × P 3N C25 46	30	226	S = M
C × C 3N C11 15	27	311	S = U^T
C × P 3N C1 1	30	281	S = M, G = DA
P × C 2N 17	32	362	S,G = U^D
C × C 3N C9 12	19	60	FA
C × P 2N C17 23	28.5	265	FA
P × C 2N C12 16	27.5	276	FA
C × C 2N C12 27	28	350	FA
C × P 3N C7 8	24	192	FA
P × C 3N C21 31	29.5	276	S = M
P × C 3N C22 33	33.5	413	S = M
Unlabeled	32.5	424	S,G = U^D
Unlabeled	32.5	391	S,G = U^D
C × C 3N C23 35	15	30	S = M
C × C 2N C18 25	28	278	S,G = U^D
P × C 2N C14 19	31	357	S = M, G = U^D
C × C 3N C19 26	27	285	NA
C × P 3N C5 6	31	299	S = M, G = M
C × C 3N 310 13	28.5	359	S = UM
P × P 3N C3 3	33.5	356	S = M
P × C 3N C24	26	191	S = M, G = M
C × P 3N C2 2	32.5	322	S = M
C × C 3N C16 21	15.5	44	S,G = U^T
Unlabeled	28.5	194	FA (near D)

[a]S = somatic tissue; G = gonadal tissue; $U^{D,T}$ = uniform ploidy, diploid or triploid; M = mosaic; DA = diploid and aneuploid; FA = fixation artifact; NA = not analyzable.
[b]C = chinook salmon; P = pink salmon.

in commercial net-pen culture and, possibly, for sea ranching in a more efficient way, compared with pure species. For example, a hybrid combining the meat quality of chinook salmon with the early seawater tolerance of the chum and pink salmon could be very desirable for the net-pen culture. Triploid interspecific hybrids frequently have been found to be more viable than the corresponding diploid hybrids (Gray et al. 1993), although this was not confirmed in these experiments. In addition, triploidization is usually associated with reproductive sterility and may reduce or eliminate the chance of natural reproduction of escaped fish. It needs to be emphasized that this study is preliminary, representing only a small fraction of fish produced and was performed on immature fish. It does not establish the ploidy of the mature animal. It is possible that selection takes place during development and modifies the ratio of mosaic cells as determined at their developmental stage examined in this report.

Materials and Methods

The objective was to produce diploid and triploid hybrids of the chinook with pink or chum salmon, and to examine ploidy of somatic and gonadal tissues, as well as their survival, seawater tolerance, and growth. For triploid hybrids, two chromosome complements are provided by the females, thus two different triploid hybrids are possible for each pair of the species. The diploid and triploid controls were also produced.

Strains of Pacific salmon were obtained at the holding facility at the National Marine Fisheries Service (NMFS), Little Port Walter Field Station in Sashin Creek, southeastern Alaska. They consisted of pink salmon, chinook salmon, and chum salmon. Gametes were collected from mature donors: chinook salmon at Sashin Creek; chum salmon and pink salmon from wild spawners at Sashin Creek and Lover's Cove Creek. Five or six members of each group at random (at the time of this study, chum were excluded as they were no longer available) were selected for ploidy examination.

Induction of Triploidy

Triploidy was induced by the heat shock of zygotes 10 min after fertilization (20 min exposure to 26°C). The survival to hatching ranged from 70–95% for diploid controls and diploid hybrids, and 40–80% for triploid forms. Fish were raised in freshwater containers, and their ploidy was assessed by flow cytometry of the blood cells. (assessment was made by an outside commercial lab and evidence for mosaicism was not requested nor determined.) The yield of triploids in random samples was 88%. Fish were subjected to seawater challenge at 1g body weight. Later, the fish were transferred to net pens and raised in salt water where sampling took place for this study.

Sampling

Progenies were sampled among diploids, triploids, and hybrids. Twenty-four juveniles were sampled for the analysis of ploidy. Whole fish were preserved in buffered 10% formalin (the abdomen was incised and opened) and shipped to the University of California, Davis, CA, (UCD). Upon arrival at UCD, the specimens were measured, weighed, and their

organs dissected for histologic examination. The decoded samples contained 9 diploids and 15 triploids (pure species and hybrids). The fork length of diploid and triploid forms were 29.2 ± 1.7 (mean ± SD) and 26.8 ± 5.8 cm, respectively. The body weights were 315 ± 42 and 243 ± 115 g. The tissues were identified and labeled with a code number upon receipt (labels for three diploid fish were missing and could not be restored). They were processed and embedded in paraffin by standard techniques and sectioned at 4 μm for histology and image cytometry.

The samples submitted for DNA ploidy analysis were coded and listed by the original label in Table 1. The results were decoded only after completion of ploidy analysis.

We had intended to analyze red blood cells (RBC), liver, kidney, spleen, and gonads but not all tissue was identifiable from each fish. In addition, five samples suffered from inadequate fixation and those results are excluded. Therefore, the results of ploidy analysis reflect only 17 of 24 sampled fish. In most cases, the somatic tissues were identified; gonadal tissue was analyzed in nine individuals. Sometimes, somatic tissue was represented by only three cell types, liver, kidney, and RBC, with RBC the only cell present in all specimens. This method of fixation and shipment of specimens is regarded as of borderline adequacy and is to be modified in future studies by having dissection of coded animals performed on site, followed by fixation and shipment to UCD.

Quantitative DNA

The tissues were stained by the modified Azure A method employed in the Cell Analysis Systems (CAS, Inc., Elmhurst, IL) kit. DNA content was integrated across the nucleus on a CAS image cytometer Model 200 using a 280-μm filter with a 20 nm bandpass, as previously described (Teplitz et al. 1990). External human diploid and tetraploid controls were used having 7.18 and 14.36 pg DNA, respectively. (The average somatic DNA content for salmonids is 5.0 pg (Johnson et al. 1987).) A minimum of 100 cells from each tissue was analyzed, distributed from each of four separated quadrants of the tissue. If a peak exceeded the G2 peak by 5% or more of cells and was in a separate DNA ploidy range from the main G1 peak (except for the G2-M peak), the population was considered to be evidence of mosaicism. Such quantitative variation never appeared in pure diploid strains.

We recognize that chromatin condensation may affect hydrolysis of DNA in the Feulgen procedure (Millett et al. 1982); however, analysis of sperm (condensed) and somatic cells (liver, etc. decondensed) yielded exact multiples in diploid and triploid fish and in human tissues, as previously reported (Pak et al. 1982).

Results

The details of the success rate of triploidization are described by Joyce et al. (1994).

The cell DNA mass was found to be around 5 pg or consistent with an even multiple or a fraction (0.5, 1.5, or 2). When the data were uncoded, the diploids, triploids, and hybrids were correlated with DNA data (Table 1). Of the 17 fish analyzed for ploidy, 11 (65%) were mosaic and these were distributed among the triploids, triploid hybrids, and one diploid hybrid. Control diploid crosses did not exhibit mosaicism.

Interestingly, 38% of the mosaics among triploids involved more than two cell lines. There were three populations of cells in these specimens, as defined by ploidy, which were either characteristic of the fish as a whole or of one or more tissues. Several kinds of triple mosaicism were encountered: diploid–triploid–tetraploid and haploid–diploid–triploid. In the former, the mechanism for production of a tetraploid from diploids is either fusion of two diploid cells or polyploidization of a diploid cell. In the latter, hexaploidy could have resulted from fusion of triploid cells; these cells could then have been reduced to diploids by multipolar mitosis. Other cytogenetic mechanisms are also possible. The cell lines containing $2N$ or greater ploidy have a reasonable expectation for survival because at least one complete diploid genome is present in each cell. The problem of survival is raised in the haploid–diploid–tetraploid mosaics, because the haploid cells do not have a complete diploid genome. It is generally understood that haploid cells exist only as end products of meiosis, the haploid gamete. There are exceptions. Certain species have haploid somatic cells or even haploid individuals, but these examples are restricted to insects. Survival of mosaic cells in salmon embryos (Miller et al. 1994) and naturally occuring hybrid salmonids (J. Seeb, personal communication) has been documented. The mechanisms for production of haploid cells are limited; they occur either from multipolar mitosis of a diploid cell or a series of nondisjunctional events. Because no intermediate ploidies were identified in our studies of salmonids or those reports of haploids in insects in the literature, we speculate that the former explanation is the more likely, although apoptosis of haploid genomes may occur. An occasional reference is made to somatic haploid cells in vertebrates and these are also mosaics (Thorne et al. 1987).

The two smallest fish were C × C triploids. All other fish were in a range of 26–33.5 cm fork length. There was no significant difference in the average body size (fork length and body weight, ANOVA, $P > 0.05$) between the diploids, triploids, and hybrid crosses (Table 2). However, the variability was much higher among triploid forms. Among the homotriploids analyzed (either C × C or P× P) four of six (67%) fish were mosaic. Of the six heterotriploids (hybrids) analyzed, all were mosaic (Table 2). These preliminary results suggest that triploidy tends to be associated with the development of mosaicism in one or more organs, but that tendency may be accentuated when the triploids are hybrids (heterotriploid). Statistical certainty is not achieved in this small sample. One diploid hybrid had somatic mosaicism, but the gonadal cells were diploid. This case was an instance of hybridization leading to mosaicism, even though both parents were diploid. Survival of haploid somatic cells may require no special adaptation. But because it is not seen in higher organisms except as terminally differentiated gametes and rarely as aberrant cells in human cancer (Oshimura et al. 1977), they may survive in combination with cells containing a complete genome through extensive intercellular commerce.

Discussion and Conclusions

Investigation of the mechanisms leading to mosaicism was not the goal of this analysis, but it might be studied in several ways. Direct examination of the chromosomes could be done by standard cytogenetic procedures. Application of strain-specific libraries of nucleotide sequences would permit

TABLE 2. Body size and proportions of individuals with somatic mosaicism among pure diploid crosses, diploid hybrids, pure triploids, and triploid hybrids of chinook and pink salmon. Data are means ± SD (*n*).

Crosses	Fork length (cm)	Body weight (g)	Mosaics %
Pure diploids	28.0±0.9 (2)	314±51 (2)	0[a]
Diploid hybrids	29.7±2.1 (4)	315±52 (4)	50
Pure triploids	24.4±7.0 (8)	209±143 (8)	67
Triploid hybrids	29.5±3.4 (7)	282±77 (7)	100

[a]Does not include two unlabeled diploid individuals (A-12, A-13, see Table 1).

a fluorescent in situ hybridization (FISH) analysis of chromosomes. It is possible that the strain contribution to hybrids and mosaics could be ascertained by FISH analysis of interphase cells. Thus, a simple sampling of blood cells with appropriately labeled probes could determine hybridization success and could be used to monitor changes in ratios among erythrocytes of mosaics. With the availability of strain-specific sequences, Southern blotting of extracted DNA could yield quantitative genomic information, albeit not chromosome-specific data. We recognize that sampling of erythrocytes may not yield information regarding mosaicism for several reasons. (1) Ploidy may vary with time in any constantly dividing tissue. (2) Flow cytometry may not detect small populations as we and others have reported (Gandour-Edwards et al. 1994; Wersto et al. 1991).

Fixation inadequacy invalidated the use of a number of specimens in this study. In reviewing the slides stained for DNA, considerable variation in quality was observed. In some cells, the sharply defined threads or "granules" of chromosomal DNA was replaced by a "ground-glass" or even a fuzzy appearance. These patterns are associated with the denaturation of DNA. This was correlated with higher coefficient of variation than normally obtained in studies of this kind (<5%). We attribute these results to one or both of two mechanisms: (1) cell death rates or DNA variation may be higher in hybrids and/or triploids because of errors of chromosome segregation and subsequent aneuploidy; and (2) inadequate fixation.

In future studies, the fixation problems will be corrected by a dissection of specimens to appropriate size and adequate fixation prior to shipment to UCD for DNA ploidy analysis. The percentage of mosaics in this study should be appreciated as preliminary. Only a fraction of the fish treated and hybridized have been examined. However, as an indication of the generality of these results in salmonids, studies being done in rainbow trout (*O. mykiss*) at UCD have yielded similar data. Diploid specimens have had uniform appropriate DNA ploidy in tissues examined. However, DNA variations and mosaicism were detected in triploids and especially in triploid × diploid crosses. These studies were not complicated by fixation artifact. (L. Perez-Carrasco, personal communication).

All of the pure strain salmonids exhibited consistent ploidy in the variety of tissues examined. However, most triploid and all triploid interspecific hybrids resulted in the formation of mosaics (up to three cell lines) involving several possible cytogenetic mechanisms. Of particular interest is the formation of viable haploid cell lines as a member of a mosaic. What effect mosaicism has on the biologic parameters of growth, fertility, and survival competence is still to be determined. However, it would appear that, although the present procedures are capable of producing triploids and hybrids, as desired, they tend toward mosaicism and, therefore, to unpredictable results. This is especially important when it involves the potential production of fertile euploid gametes in artificially produced triploid fish, as well as in diploid and triploid hybrids of fish and amphibians (Cherfas et al. 1984; Van Enennaam et al. 1990; Ohtani 1993). However, whether mosaicism persists or quantitatively varies during development is yet to be established. These preliminary results suggest that further, more complete studies are necessary to resolve remaining questions.

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Saltwater Exposure at Fertilization Induces Ploidy Alterations, Including Mosaicism, in Salmonids[1]

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We found that salt water induces ploidy alterations in salmonid embryos. Flow cytometry analysis revealed significantly higher frequencies of haploids, triploids, heteroploid mosaics, and aneuploids in rainbow trout (*Oncorhynchus mykiss*) embryos experimentally exposed to salt water from fertilization to the two- and eight-cell stages of development. Heteroploid mosaics have been reported in diploid and triploid salmonid hybrids, although none were observed in the triploid coho salmon or diploid and triploid coho salmon (*O. kisutch*) × chinook salmon (*O. tshawytscha*) hybrids we examined. No mosaics were observed in intertidally spawned pink salmon (*O. gorbuscha*) embryos. Salt water could induce ploidy alterations by causing chromosome segregation errors during meiosis, mitosis, or both. Heteroploid embryos appeared morphologically normal although they may possess physiological deficiencies not immediately apparent.

Les auteurs ont découvert que l'eau de mer provoque des modifications du degré de ploïdie chez les embryons de salmonidés. Une analyse par cytométrie de flux a révélé des fréquences nettement plus élevées d'haploïdes, de triploïdes, d'aneuploïdes et de mosaïques hétéroploïdes chez des embryons de truite arc-en-ciel exposés à de l'eau de mer à partir de la fécondation jusqu'aux stades de deux et de huit cellules. Des mosaïques hétéroploïdes ont été signalées chez des saumons hybrides diploïdes et triploïdes, bien qu'aucune n'ait été observée chez les cohos triploïdes et les hybrides diploïdes et triploïdes obtenus de croisements de saumons cohos et de saumons quinnats que les auteurs ont examinés. En outre, ils n'ont observé aucune mosaïque chez les embryons de saumon rose issus du frai dans la zone intertidale. Il se peut que l'eau de mer provoque des modifications du degré de ploïdie en causant des erreurs de ségrégation des chromosomes lors de la méïose, de la mitose ou des deux. Les embryons hétéroploïdes semblent normaux au plan morphologique, bien qu'ils puissent avoir des déficiences physiologiques qui ne sont pas tout de suite évidentes.

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Data collected during the damage assessment of the March 1989 Exxon Valdez oil spill showed elevated mortalities of pink salmon (*Oncorhynchus gorbuscha*) embryos in the intertidal areas of oil-contaminated streams. These mortalities appear to be persisting through subsequent generations, even though oil contamination is greatly diminished (Sharr et al. 1994). One of our major foci has been to examine the relationship between embryo mortality and occurrence of stream oiling. We are also, in cooperation with the National Marine Fisheries Service, Auke Bay Laboratory, Juneau, Alaska, studying the potential of crude oil to induce genetic damage in pink salmon embryos and larvae. As a part of the latter study we screened oil-exposed pink salmon embryos for genetic damage using flow cytometry, a technique shown to be rapid and effective for detection of DNA macrolesions (McBee and Bickham 1988; Bickham 1990; Lamb et al. 1991).

[1]Contribution PP-086 of the Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, Juneau.

The controlled oiling of embryos was done at the Little Port Walter Laboratory (LPW), Baranof Island, Alaska, in a simulated intertidal environment. Incubation of these embryos was designed to emulate the fluctuating seawater–freshwater environment experienced by pink salmon embryos residing in the intertidal areas affected by the Exxon Valdez oil spill.

During our initial analysis of these test embryos we observed triploids and heteroploid mosaics (individuals possessing two or more cell populations of differing ploidy) (Fig. 1). These individuals were equally distributed across all exposure groups, including unoiled controls, and therefore were clearly not correlated with oil exposure (Table 1).

We were immediately interested in determining what factor caused the ploidy alterations, especially mosaicism, in these pink salmon embryos. Aneuploid and heteroploid mosaics, although rare, have been observed in triploid conspecifics and diploid and triploid hybrids in salmonids (Refstie et al. 1977; Allen and Stanley 1978; Smith and Lemoine 1979; Ma and Yamazaki 1986; Goodier et al. 1987), but we were not aware of previous observations of such mosaics in conspecific diploids. We were informed that salt

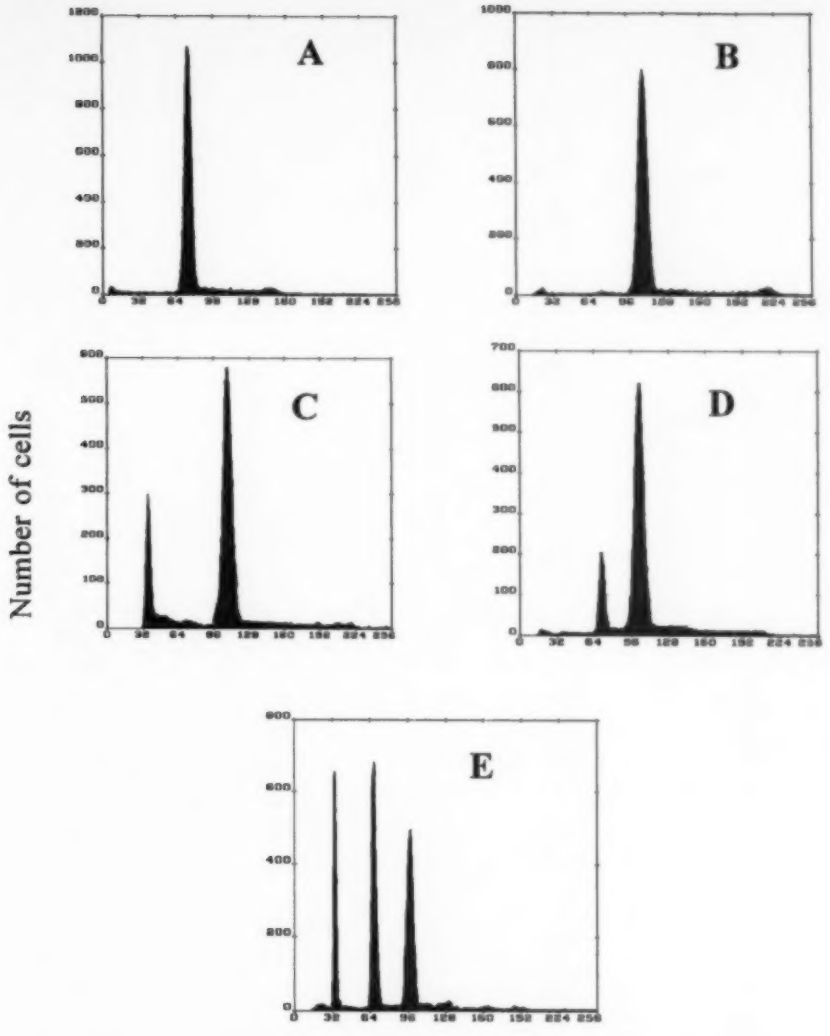

FIG. 1. DNA histograms observed during flow cytometry analysis of Little Port Walter oil exposed pink salmon embryos. (A) Normal diploid; (B) triploid; (C) haploid–triploid mosaic; (D) diploid–triploid mosaic; (E) haploid–diploid–triploid mosaic. DNA content is indicated along the horizontal axis and number of cells along the vertical axis.

water may have been introduced into the incubators by the fluctuating seawater system at LPW during egg fertilization. Therefore, we designed this study to include a test for the potential of salt water to induce ploidy alterations in salmonid embryos.

First, we screened triploid coho salmon (*O. kisutch*), and diploid and triploid coho salmon × chinook salmon (*O. tshawytscha*) hybrids to estimate the frequency of heteroploids occurring in modified salmonid genomes. Secondly, we conducted an extensive screening of intertidally spawned pink salmon fry from Prince William Sound, Alaska, to determine if triploids and heteroploid mosaics were a naturally occurring phenomenon associated with intertidal spawning (and possibly a causal agent of the embryo mortality observed in this region). Finally, we conducted a controlled exposure of rainbow trout (*O. mykiss*) eggs and embryos to saltwater during the interval from fertilization to the two-cell stage and from fertilization to the eight-cell stage to directly assess the potential of saltwater exposure to induce ploidy alterations.

No heteroploids were observed in the triploid coho salmon or in the diploid or triploid coho salmon × chinook salmon hybrids. Interestingly, one triploid but no heteroploids were observed in the intertidally spawned pink salmon. However, triploids and heteroploids similar to those observed in the LPW pink salmon were observed in rainbow trout exposed

TABLE 1. Ploidies observed during flow cytometry analysis of Little Port Walter Laboratory pink salmon embryos and larvae. Embryos were exposed to six oil levels in a controlled experiment at LPW. Triploid and heteroploid individuals were distributed approximately equally across all treatment groups, including unoiled controls, and therefore were not correlated with oil exposure.

	Oil exposure						
Ploidy	Control (0.0 g/kg)	Light 1 (0.1 g/kg)	Light 2 (0.4 g/kg)	Moderate (1.5 g/kg)	Heavy 1 (5.7 g/kg)	Heavy 2 (6.1 g/kg)	Total
Diploid	173	160	155	133	44	35	700
Triploid	4	3	2	0	1	2	12
Haploid–Triploid	1	2	3	1	0	1	8
Diploid–Triploid	1	0	0	1	0	0	2
Haploid–Diploid–Triploid	0	0	0	0	0	1	1
Total	179	165	160	135	45	39	723
% Nondiploid	3.35 (6/179)	3.03 (5/165)	3.13 (5/160)	1.48 (2/135)	2.20 (1/45)	10.26 (4/39)	3.18 (23/723)

to salt water. It is likely, therefore, that the triploids and heteroploids observed in the LPW pink salmon were induced by saltwater exposure at fertilization.

Materials and Methods

Flow Cytometry

All samples used for flow cytometry analysis in the various experiments were randomly chosen and analyzed as preparations of whole embryos or larvae. Embryos were first dissected from the egg by removing the chorion. The yolk sac was removed from embryos and larvae using forceps and the remaining tissue gently blotted to remove any residual yolk material.

Whole embryos and larvae were placed into 1.5-mL microcentrifuge tubes containing 1 mL of nuclear isolation medium (NIM; 0.9% NaCl, 10 mM Tris, 2 mM CaCl2, 2 mM $MgCl_2$, 0.1% NP40, 106 mM $MgSO_4$, and 1 mg/100 mL DAPI (4,6-diamidino-2-phenylindole dihydrochloride)) to isolate and stain cell nuclei. Tissues were minced with scissors, allowed to incubate at 2–3°C for 15 min, and filtered through a 70-μm Nitex nylon filter. These stained nuclear suspensions were refrigerated overnight at 3°C. Samples were triturated three times through a 26-gauge syringe and filtered through a 41-μm Nitex nylon filter immediately prior to flow cytometry analysis.

Samples were randomly analyzed in a blind fashion using a Partec Pas II flow cytometer with optical filters for DAPI excitation and Acqcyte data acquisition and Multicycle DNA analysis software for cell cycle histogram generation (Phoenix Flow Systems, San Diego, CA). The operator was not made aware of the treatments until after analysis to prevent bias. Histograms were evaluated for the presence of cell populations possessing abnormal quantities of DNA, which appeared as peaks distinct from the normal G_1 peak (diploid peak containing interphase and early prophase cells). Heteroploids were those histograms displaying an additional peak (or peaks) distinct from the diploid G_1 peak, which contained at least 10% of the total events collected. Ploidy was assigned to distinct cell populations based on their DNA Index (DI = mean channel number of abnormal G_1 peak/mean channel number of diploid G_1 peak). DIs of 0.5, 1.0, and 1.5 corresponded to haploid, diploid, and triploid populations, respectively.

Calf thymus nuclei or trout erythrocyte nuclei (Riese Enterprises, Inc., San Jose, CA) were used as an external standard to calibrate the flow cytometer prior to each sampling session and after every sixth sample during sessions. Gain controls were used to correct for any drift observed in the standard. A distilled water blank was run between samples to remove any residual material from the preceding sample. The coefficient of variation of the DAPI stained standard was routinely 1.9–2.5. Samples were analyzed at flow rates of 500 nuclei/s with a total of 20 000 nuclei collected per sample.

Triploids and Triploid Hybrids

Triploid coho salmon and diploid and triploid coho salmon female × chinook salmon male hybrids (larvae) were obtained from the Alaska Department of Fish and Game Broodstock Development Center (BDC) in Anchorage. Triploidy had been induced the previous fall by exposing newly fertilized eggs to a 26°C heat shock. One hundred heat-shocked coho salmon, 50 heat-shocked coho salmon × chinook salmon hybrids, and 35 non-heat-shocked coho salmon × chinook salmon hybrids were screened for the presence of heteroploids in February 1993.

Prince William Sound Pink Salmon Larvae

Pink salmon larvae were collected from the gravel in 16 streams around Prince William Sound during March and April 1993. The intertidal portions of eight of these streams had been contaminated by crude oil. Approximately 200 larvae were collected from both the upstream (above mean high tide) and intertidal (1.8–3.7 m above mean low tide) locations in five streams and from intertidal locations only in 11 streams. Larvae were shipped live to our laboratory and maintained in individual lots until flow cytometric analysis. Twenty larvae were processed from each of the upstream locations and 11 of the intertidal locations. Twenty-four larvae were processed for each of the remaining intertidal locations.

Rainbow Trout Saltwater Exposure

We conducted two experiments to investigate the frequency of polyploids and heteroploids occurring in salmonids exposed to salt water during early embryogenesis. Pink

TABLE 2. Ploidies observed during flow cytometry analysis of rainbow trout embryos from experiment 1. Embryos were exposed to four concentrations of salt water from fertilization to the two-cell stage. Unreadable DNA content histograms were classified as unknown and were not used to calculate the percentage of nondiploid individuals.

	Salt Water concentration				
Ploidy	Control (0 ppt)	Low (8 ppt)	Medium (16 ppt)	High (32 ppt)	Total
Diploid	46	42	29	14	131
Haploid	0	1	7	2	10
Triploid	0	1	1	0	2
Haploid–Diploid	0	1	0	0	1
Diploid–Triploid	0	0	1	0	1
Haploid–Diploid–Triploid	0	1	0	0	1
Aneuploid	0	0	1	0	1
Unknown	7	6	12	33	58
Total	53	52	51	49	205
% Nondiploid	0.00 (0/46)	8.70 (4/46)	25.64 (10/39)	12.50 (2/16)	10.88 (16/147)

TABLE 3. Ploidies observed during flow cytometry analysis of rainbow trout embryos from experiment 2. Embryos were exposed to four concentrations of salt water from fertilization to the eight-cell stage. Unreadable DNA content histograms were classified as unknown and were not used to calculate the percentage of nondiploid individuals.

	Salt Water concentration				
Ploidy	Control (0 ppt)	Low (8 ppt)	Medium (16 ppt)	High (32 ppt)	Total
Diploid	32	26	14	1	73
Haploid	1	1	6	3	11
Triploid	1	0	0	0	1
Haploid–Diploid	0	2	0	0	2
Diploid–Triploid	0	0	2	0	2
Haploid–Diploid–Triploid	0	0	0	0	0
Aneuploid	0	1	3	0	4
Unknown	0	3	6	29	38
Total	34	33	31	33	131
% Nondiploid	5.88 (2/34)	13.33 (4/30)	44.00 (11/25)	75.00 (3/4)	21.51 (20/93)

salmon eggs were not available in April 1993, so rainbow trout eggs were used. Embryos were exposed to salt water from fertilization to the two-cell stage in experiment 1 and from fertilization to the eight-cell stage in experiment 2. Refstie et al. (1977) found high frequencies of polyploids and mosaics in Atlantic salmon (*Salmo salar*) embryos exposed to cytochalasin B up to the two-, four-, and eight-cell stages with the highest frequencies occurring in eight-cell exposure groups. We were interested in determining if duration of saltwater exposure had a similar effect on polyploid and heteroploid frequency in rainbow trout.

Rainbow trout gametes were obtained from the BDC. Eggs from individual females were placed in 1-L plastic bags; sperm from individual males was placed in 15-mL plastic centrifuge tubes. Gametes were transported back to the Genetics Laboratory in insulated coolers containing wet ice. Experiments were conducted the same day of gamete collection.

Experiment 1, two-cell saltwater exposure, was conducted on April 1, 1993. Eggs from 10 females and sperm from 12 males were pooled prior to fertilization and chilled on wet ice in coolers throughout the duration of the experiment. Four levels of saltwater concentration; 0 ppt, 8 ppt, 16 ppt, and 32 ppt (corresponding to saltwater concentrations of 0%, 25%, 50%, and 100%, respectively), were used for activation and embryo exposure. All saltwater solutions were made from the same 32-ppt stock solution diluted with fresh water to the appropriate concentration. Salinities were verified using a refractometer (BioMarine, Inc., Hawthorne, CA). All solutions were maintained at 7°C.

Twenty-five millilitres of eggs (approx. 250 eggs) were measured into each of four 100-mL paper cups, one cup for each saltwater treatment. Eggs were fertilized with 100 μL of sperm and immediately activated with 50 mL of treatment water. Each cup was gently swirled two times and allowed to fertilize for one min. The activation water was decanted and the eggs gently rinsed once with 50 mL of treatment water. Eggs were gently poured in 70 × 70 mm plastic cups with screened bottoms. Each cup was placed in one of four 26 × 19 × 10 cm plastic trays containing 1 L of 7°C treatment water. This process was replicated 10 times with each of the four saltwater treatments being randomly assigned

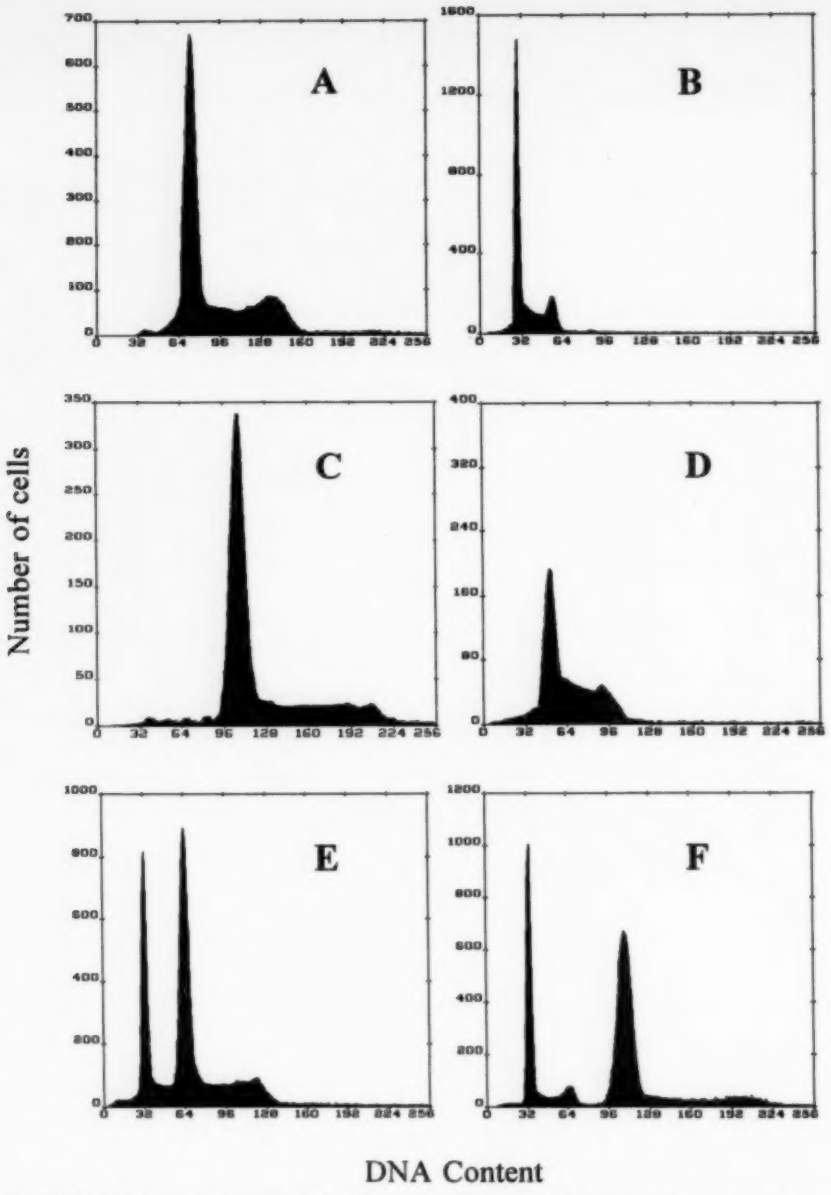

FIG. 2. DNA histograms observed during flow cytometry analysis of rainbow trout embryos from saltwater exposure experiments 1 and 2. (A) Normal diploid; (B) haploid; (C) triploid; (D) aneuploid; (E) haploid–diploid mosaic; (F) haploid-triploid mosaic. DNA content is indicated along the horizontal axis and number of cells along the vertical axis.

to one of the cups in each replicate. Eggs were allowed to incubate in treatment water for 8 h (56 hour-degrees). The number of cell divisions was confirmed by microscopic examination of eggs cleared in Stockards' solution. Egg cups were transferred after treatment to Heath trays in a 7°C freshwater recycling system for incubation.

Experiment 2, eight-cell saltwater exposure, was conducted on April 9, 1993, and was similar to experiment 1 with the following exceptions: (1) eggs from six females and sperm from eight males were used, (2) fertilizations were replicated six times, and (3) eggs were allowed to incubate in treatment water for 26 h (182 hour-degrees).

Embryos were scored for malformations and prepared for flow cytometry analysis 22 d after fertilization. Randomly sampled embryos were examined under 10× magnification prior to removal from the egg and scored as appearing normal or malformed. Eggs that had not developed to embryos were scored and discarded. The criteria used to classify

TABLE 4. Total number of normal and abnormal embryos observed during flow cytometry analysis of saltwater-exposed rainbow trout from experiments 1 and 2.

Ploidy	Embryo appearance		
	Normal	Abnormal	Total
Diploid	190	14	204
Haploid	12	9	21
Triploid	2	1	3
Haploid–Diploid	2	1	3
Diploid–Triploid	3	0	3
Haploid–Diploid–Triploid	1	0	1
Aneuploid	0	5	5
Unknown	8	88	96
Total	218	118	336

embryos as malformed included deformity of the head or spine, an abnormally small embryo, or presence of an undifferentiated cell mass.

Differences in the frequencies of diploids, haploids, triploids, mosaics, and aneuploids between the treatment groups were evaluated using a chi-square test of homogeneity ($\alpha = 0.05$). Correlation between embryo appearance and ploidy was calculated using Ives and Gibbons' (1967) correlation for dichotomous nominal data (r_n) with significance determined by a chi-square test of independence ($\alpha = 0.05$).

Results

Triploids and Triploid Hybrids

No heteroploids were observed in any of the triploid coho salmon, diploid coho salmon × chinook salmon hybrids, or triploid coho salmon × chinook salmon hybrids examined. All non-heat-shocked coho salmon × chinook salmon hybrids were diploid; 70% (70/100) of the heat-shocked coho salmon and 96% (48/50) of the heat-shocked coho salmon × chinook salmon hybrids were triploid.

Prince William Sound Pink Salmon Larvae

No heteroploids were observed in the five upstream locations (100 larvae examined) or the 16 intertidal locations (340 larvae examined). One triploid individual was observed in an intertidal sample.

Rainbow Trout Saltwater Exposure

Ploidy alterations were seen in experiments 1 and 2 and included haploid, triploid, and heteroploid individuals (Tables 2 and 3). Five embryos with aneuploid G_1 DNA contents (DI = 0.710 ± 0.026; mean ± SD) were observed. Heteroploids were mosaics of haploid–diploid, diploid–triploid, and haploid–diploid–triploid cell populations (Fig. 2) with haploid cells predominating in haploid–diploid mosaics and triploid cells predominating in diploid–triploid mosaics. Samples were classified as unknown when a G_1 peak was not discernable and were excluded from subsequent statistical analysis. Unknowns probably occurred when too few cells were present in a sample to generate an adequate cell cycle histogram, as might occur if the embryo remained an undifferentiated blastula.

The total frequency of haploid, triploid, heteroploid, and aneuploid individuals differed significantly between the control and saltwater groups in both experiments ($P = 0.002$ for experiment 1; $P = 0.001$ for experiment 2). The frequency of heteroploids did not significantly differ between groups ($P = 0.456$ for experiment 1; $P = 0.289$ for experiment 2).

One haploid individual and one triploid individual were observed in the control group of experiment 2.

The correlation between ploidy and embryo appearance was significant ($r_n = 0.76$; $P < 0.001$) (Table 4). Embryos with unknown ploidies were classified according to appearance but were not included in the correlation calculation. Diploid, triploid, and heteroploid embryos were more likely to appear normal; aneuploid embryos were all abnormal; and haploid embryo appearance was nearly equally distributed. All but eight embryos of unknown ploidy were abnormal.

Discussion

The inference that the high frequencies of heteroploid mosaics and triploids observed in the LPW pink salmon embryos were induced by saltwater exposure was supported by these experiments. Increased frequencies of nondiploid individuals, including mosaics similar to those in the LPW pink salmon, were observed in rainbow trout embryos exposed to salt water at fertilization. Therefore, the occurrence of mosaics and triploids in the LPW pink salmon embryos may have been enhanced by the salt water introduced into the incubation system during egg fertilization and early cell divisions.

The possibility that salt water may induce mosaicism is intriguing. Several chemical and physical treatments designed to induce polyploidy in salmonids also cause mosaicism. Heteroploid mosaics are induced in brook trout (*Salvelinus fontinalis*) by cholchicine exposure (Smith and Lemoine 1979) and in Atlantic salmon and rainbow trout by cytochalasin B exposure (Refstie et al. 1977; Allen and Stanley 1979; Refstie 1981). Heteroploidy is induced in brook trout by cold shock (Lemoine and Smith 1980), in rainbow trout by heat shock (Chourrout and Nakayama 1987), and in chum salmon (*O. keta*) and masu salmon (*O. masou*) by pressure shock (Yamazaki and Goodier 1993).

The potential mechanisms involved in heteroploid production are complex. The treatments listed above induce chromosome segregation errors during meiosis or mitosis by disrupting spindle formation (cholchicine), inhibiting cytokinesis (cytochalasin B), or both (temperature and pressure). Salt water may enhance heteroploid production by causing chromosome segregation errors as well, but it could also induce mosaicism by mediating polyspermy or a combination of the two.

Meiosis II is easily disrupted in salmonids, either spontaneously (Thorgaard et al. 1982) or by various chemical and physical treatments (reviewed in Seeb and Miller 1990). Such meiotic disruptions produce triploids by inducing incorporation of the second polar body into the zygote. Meiotic errors alone could generate haploid–diploid mosaics without subsequent mitotic errors. Diploid and haploid cells might both be generated if karyogamy occurred followed by independent development of the second polar body.

Given a diploid or triploid zygote, saltwater-induced mitotic errors might also generate mosaics. Chromosome lagging, nondisjunction, or mono-, tri-, or tetra-polar division

all contribute to mitotic errors (Ford and Correll 1992) and have been observed in salmonid hybrids and pressure-shocked masu salmon embryos (Yamazaki et al. 1989; Yamazaki and Goodier 1993). Mitotic segregation errors involving haploid genome complements might lead to haploid or diploid subpopulations of cells within a diploid or triploid individual.

In salmonids, polyspermy, the entrance of more than one sperm through the micropyle, is prevented by micropyle closure during water hardening (the influx of water across the chorion forming the perivitelline space) (Kobayashi and Yamamoto 1993). Water hardening proceeds rapidly after fertilization but is retarded by the presence of salt water (Black 1951). Therefore, salt water may delay micropyle closing promoting polyspermy and the production of triploids or mosaics. Polyspermy is the most likely origin of heteroploid mosaic chickens (Thorne et al. 1987) and has been induced in salmonids (Fields 1991)

We expected a higher frequency of nondiploid rainbow trout in saltwater experiment 2 than in experiment 1. Our assumption was that more cell divisions occurring in the presence of salt water would increase the probability of an error during mitosis. However, this was not observed. Data in Refstie et al. (1977) suggest that susceptibility to ploidy alterations may be species specific. In their experiment rainbow trout responded to cytochalasin B treatments up to the four-cell stage but not beyond; in contrast, the frequency of polyploids and mosaics in Atlantic salmon increased after an eight-cell exposure. Salt water may only disrupt meiosis II and the first cell division in rainbow trout; therefore, exposures beyond the two-cell stage would have no effect on ploidy. Again, the triploid individual observed in the control treatment of experiment 2 was not surprising; spontaneous triploidy does occur in hatchery rainbow trout (Thorgaard et al. 1982).

The absence of heteroploids in intertidally spawned pink salmon embryos may be explained by the timing of saltwater exposure. In Prince William Sound, as much as 75% of returning pink salmon may spawn intertidally, and most are expected to spawn at low tide (Helle et al. 1964). Eggs deposited at low tide would be fertilized in fresh water and may not be exposed to saltwater for some time. By that time meiosis II and perhaps the first mitotic division would have completed. Water hardening would also have occurred rendering the chorion less permeable to salt (Weisbart 1968). Together these factors would make alterations of ploidy less likely in the developing zygote.

The lack of correlation between malformed individuals and heteroploidy also is not surprising. The evolution of salmonids from tetraploid ancestors (Allendorf and Thorgaard 1984) has resulted in a high tolerance of polyploidy within this taxon, which is reflected in the ease with which viable triploids, tetraploids, and triploid interspecific hybrids can be artificially produced (Seeb and Miller 1990). Although heteroploid individuals are not distinguishable based on external morphology, they may possess physiological or biochemical deficiencies that are not immediately apparent. The question of whether mosaicism occurs in all tissues or is tissue-specific remains. Future flow cytometry analysis of individual tissues (e.g., liver, gonad, kidney, gill) from saltwater-exposed individuals is planned. This should identify the location of haploid or polyploid cells and provide insight into their possible effect on the organism.

Acknowledgements

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Genetic Relationships Among Chum Salmon Populations in Southeast Alaska and Northern British Columbia

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Allozymes from 46 loci were analyzed from chum salmon (*Oncorhynchus keta*) collected at 61 locations in southeast Alaska and northern British Columbia. Of the 42 variable loci, 21 had a common allele frequency <0.95. We observed significant heterogeneity within and among six regional groups: central southeast Alaska, Prince of Wales Island area, southern southeast Alaska – northern British Columbia, north-central British Columbia, and two groups in the Queen Charlotte Islands. Genetic variation among regions was significantly greater than within regions. The three island groups were distinct from each other and from the mainland populations. Allele frequencies were stable over time in 14 of 15 locations sampled for more than 1 yr. The geographic basis for heterogeneity among regions is confounded in part by spawning-time differences. The Prince of Wales and Queen Charlotte populations spawn in the fall; the mainland populations spawn mainly in the summer, although some overlap exists. Overall, most genetic diversity (97%) occurred within sampling locations; the remaining diversity was distributed almost equally within and among regions. Our genetic data may provide fishery managers a means to estimate stock composition in the mixed-stock fisheries near this boundary between the United States and Canada.

Les auteurs ont analysé les allozymes en 46 loci chez des saumons kétas (*Oncorhynchus keta*) prélevés à 61 sites du sud-est de l'Alaska et du nord de la Colombie-Britannique; des 42 loci variables, 21 possédaient une fréquence allélique commune <0,95. Ils ont observé une hétérogénéité significative tant au sein qu'entre les six groupes régionaux suivants : sud-est central de l'Alaska, région de l'Île Prince-William, secteur sud du sud-est de l'Alaska – nord de la Colombie-Britannique, nord-central de la Colombie-Britannique et deux des Îles de la Reine-Charlotte. La variation génétique entre les régions était nettement plus élevée que la variation au sein des régions, tandis que les trois groupes insulaires étaient distincts l'un de l'autre et des populations continentales. Les fréquences alléliques étaient stables dans le temps à 14 des 15 sites échantillonnés pendant plus d'un an. Le fondement géographique de l'hétérogénéité entre les régions est obscurci en partie par des différences liées à la saison de fraie. Les populations de l'Île Prince-William et des îles de la Reine-Charlotte frayent en automne tandis que les populations continentales frayent surtout en été, bien qu'un certain chevauchement existe. En général, la plus grande partie de la diversité génétique (97%) a été notée dans les sites d'échantillonnage tandis que le reste était réparti également entre les régions et dans les régions. Les données génétiques recueillies permettront peut-être aux gestionnaires des pêches d'estimer la composition des stocks faisant l'objet d'une pêche mixte près de la frontière canado–américaine.

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Chum salmon (*Oncorhynchus keta*) spawn in hundreds of streams in southeast Alaska and northern British Columbia. With few exceptions, these chum salmon are wild stocks not enhanced or manipulated by people. They are a major resource in this region, caught in a number of fisheries on both sides of the United States – Canada border. Several fisheries that harvest other species of salmon incidentally catch a substantial number of chum salmon (Clark et al. 1986). Concern about the conservation of chum salmon stocks in the boundary area has arisen because recent annual escapements in this area have been well below target levels or historic averages (Pacific Salmon Commission 1987).

Tagging studies initiated because of this concern have shown that returning adult chum salmon come into the coastal waters between southeast Alaska and northern British Columbia as mixed stocks (Alaska Department of Fish and Game 1986). Generally, they arrive along the southern coast of southeast

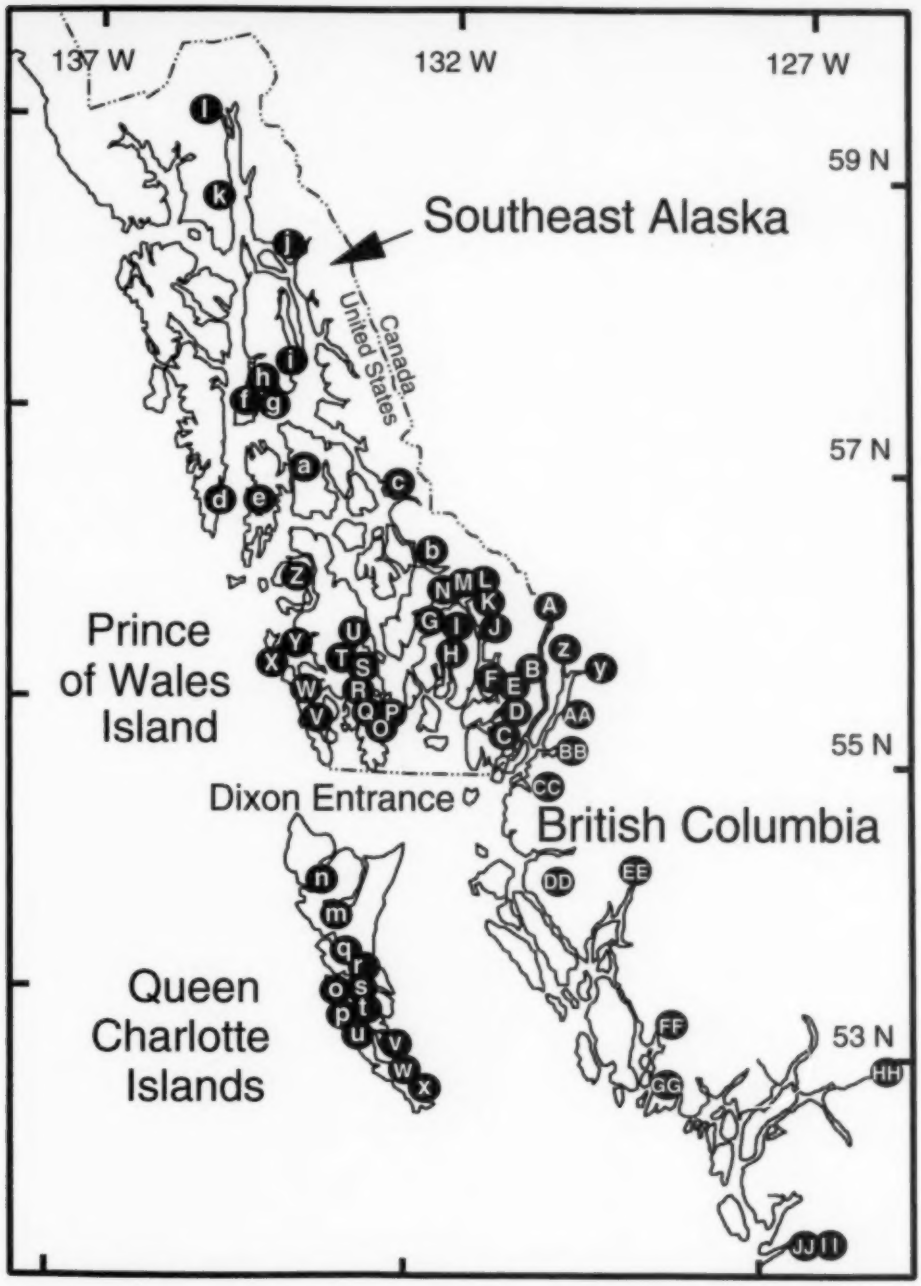

FIG. 1. Locations of chum salmon samples collected in southeast Alaska and northern British Columbia. Letters correspond to location names in Table 1 and Figs. 2 and 3.

Alaska and move into Dixon Entrance before swimming north or south toward their spawning streams. Spawning time, age structure, morphology, and developmental differences have been documented for chum salmon populations throughout their range (Bakkala 1970; Helle 1984; Beacham et al. 1987, 1988; Salo 1991). Several studies have also shown significant genetic variation among chum salmon populations (Okazaki 1981, 1982, 1983; Davis and Olito 1982; Beacham et al. 1985, 1987; Phelps et al. 1994; R. Wilmot, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801, personal communication). These studies sampled populations from different areas of the chum salmon range or examined a relatively small number of loci. Except for several populations sampled with a limited number of loci (Davis and Olito 1982), the genetic structure of chum salmon populations in southeast Alaska has not been investigated.

TABLE 1. Group designation (letters correspond to streams listed in Figs. 1–3, Appendix 1), location, date of collection, and numbers of chum salmon samples used for electrophoretic analysis. Districts are Alaska Department of Fish and Game Statistical Areas. Areas are Canadian Statistical Areas. *N* is number of samples.

Group designation	Location	Date	*N*
	Southeast Alaska		
District 101: Behm Canal – Portland Canal – Boca de Quadra			
A	Fish Creek	5 Sept. 1986	100
		26 Aug. 1987	50
		8 Aug. 1988	100
		25 Sept. 1988	52
B	Tombstone River	10 Aug. 1986	98
C	Marten River	9 Aug. 1986	105
D	Keta River	25 July 1986	101
E	Blossom River	26 July 1986	101
F	Wilson River	8 Aug. 1986	103
G	Traitor's Creek	20 Aug. 1986	101
		22 Aug. 1988	105
H	Carroll River	19 Aug. 1986	100
I	Portage Creek	30 July 1986	111
		22 Aug. 1988	63
J	King Creek	20 July 1986	100
		20 July 1988	52
K	Klahini River	12 Aug. 1986	102
L	Eulachon Creek	13 Aug. 1986	90
M	Grant Creek	14 Aug. 1986	127
N	Herman River	28 July 1986	100
District 102: eastern Prince of Wales Island			
O	Kugel Creek	23 Sept. 1986	104
P	Aiken Creek	22 Sept. 1986	100
Q	Disappearance Creek	21 Sept. 1986	100
		21 Sept. 1988	100
R	Lagoon Creek	20 Sept. 1986	102
S	Old Tom Creek	24 Sept. 1986	100
		22 Sept. 1988	53
T	Cabin Creek	26 Sept. 1986	103
U	Karta River	25 Sept. 1986	100
District 103: western Prince of Wales Island area			
V	Coco Harbor	24 Sept. 1987	100
W	Breezy Bay	12 Sept. 1986	100
		22 Sept. 1987	100
		5 Sept. 1988	54
X	Port Real Marina	10 Sept. 1986	100
		9 Sept. 1988	48
Y	Cruz Cove	8 Sept. 1986	84
		26 Sept. 1987	56
		3 Sept. 1988	54
Z	Tokeen Creek	9 Sept. 1986	101
District 105: western Sumner Strait			
a	Irish Creek	24 Aug. 1986	98
District 107: Ernest Sound – Bradfield Canal			
b	Harding River	22 Aug. 1986	95
District 108: Stikine River			
c	North Arm Creek	10 Aug. 1987	81
District 109: Southern Chatham Strait – western Frederick Sound			
d	Lover's Cove Creek	19 Aug. 1987	100
		16 Aug. 1990	31
e	Elena Bay	25 Aug. 1989	61
f	Eliza Harbor	27 Aug. 1989	61
District 110: Eastern Frederick Sound			
g	Donkey Creek	25 Aug. 1986	100
		25 Aug. 1990	62
h	Pybus Bay east	28 Aug. 1989	65
i	Gambier Bay southwest	29 Aug. 1989	27
		25 Aug. 1990	40
District 115: Lynn Canal			
k	Lynn Brothers	28 July 1988	50
l	Herman Creek	17 Oct. 1987	100
		22 Sept. 1990	59
	North-central British Columbia		
Area 1: northern Queen Charlotte Islands			
m	Awun River	19 Oct. 1989	75
Area 2: western Queen Charlotte Islands			
n	Mace Creek	mid-Oct. 1989	100
o	Peel Creek	11 Oct. 1989	75
p	Tasu Creek	11 Oct. 1989	100
Area 2: eastern Queen Charlotte Islands			
q	Lagins Creek	12 Oct. 1989	91
r	Deena River	10 Oct. 1989	82
s	Pallant Creek	9 Oct. 1988	100
t	Lagoon Creek	mid-Oct. 1989	83
u	Salmon River	20 Oct. 1989	57
v	Sedgewick Creek	mid-Oct. 1989	74
w	Bag Harbor Creek	mid-Oct. 1989	89
x	Surprise Creek	mid-Oct. 1989	85
Area 3Z: Portland and Observatory Inlets – Nass River			
y	Kshwan River	13 Sept. 1988	88
z	Kitsault River	11 Aug. 1988	95
AA	Stagoo Creek	11 Aug. 1988	75
		18 Sept. 1989	53
BB	Khutzeymateen River	13 Sept. 1988	94
CC	Toon River	12 Sept. 1988	98
Area 4: Skeena River area			
DD	Ecstall River	12 Sept. 1988	99
Area 6			
EE	Kitimat River	6 Aug. 1988	92
		8 Aug. 1989	100
Area 7			
FF	Mussel River	27 Sept. 1989	31
GG	Neekas Creek	26 Sept. 1989	54
Area 8: Bella Coola River			
HH	Klownik Creek	31 Aug. 1989	100
Area 10			
II	Nekite Channel	15 Sept. 1989	100
JJ	Nekite River	30 Sept. 1989	97

We collected and analyzed samples from chum salmon throughout southeast Alaska and northern British Columbia to expand basic knowledge of their genetic population structure. Understanding the genetic variation may be useful to identify stocks in mixed-stock fisheries, to evaluate hatchery and wild stock interactions, and to protect wild stock genetic diversity. The present population structure may also provide insight into the postglacial dispersion of chum salmon in southeast Alaska and northern British Columbia.

Methods and Materials

Tissue samples were collected from adult chum salmon in 61 spawning locations in southeast Alaska and northern British Columbia between 1986 and 1990 (Fig. 1, Table 1). Several locations were sampled in more than one year to examine year-to-year differences. Muscle, heart, liver, and eye

TABLE 2. Protein coding loci (Shaklee et al. 1990) for enzymes resolved in this study and the tissues and buffers in which they were resolved. Peptidase loci are also designated according to their substrate specificity. L, liver; H, heart; M, muscle; E, eye.

Enzyme	EC No.	Locus	Tissue (buffer[a])
Aconitate hydratase	4.2.1.3	*mAH-1,2**	H and L (B)
		*mAH-3**	H and L (B)
		*sAH**	L (B)
Alanine aminotransferase	2.6.1.2	*ALAT*[b,c]	M (H)
Aspartate aminotransferase	2.6.1.1	*mAAT-1*[b,c]	M (A), H (B)
		sAAT-1,2[b,c]	M (E), H (D)
		sAAT-3[b,c]	E (G)
Creatine kinase	2.7.3.2	*CK-A1*[c]	M (G)
		CK-A2[c]	M (G)
		CK-B[b,c]	E (J)
		*CK-C1**	E (J)
		*CK-C2**	E (J)
Esterase-D	3.1.-.-	*ESTD-1*[b,c]	M and H (H)
Fumarate hydratase	4.2.1.2	*FH*[b,c]	M (E), E (F)
Formaldehyde dehydrogenase (glutathione)	1.2.1.1	*FDHG*[c]	H and L (B)
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A*[b,c]	M and H (G)
		GPI-B1,2[b]	M and H (G)
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1*[c]	M (I), H (D)
		G3PDH-2[b,c]	H (D)
		G3PDH-3[b,c]	H (D)
L-Iditol dehydrogenase	1.1.1.14	*IDDH-1*[c]	H (G)
		IDDH-2[b,c]	L (G)
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	*mIDHP-1*[b,c]	M (A)
		mIDHP-2[c]	M (A)
		*sIDHP-2**	L (B)
L-Lactate dehydrogenase	1.1.1.27	*LDH-A1*[b,c]	M (G)
		LDH-A2[c]	M (G)
		LDH-B1[c]	M, H, and E (G)
		LDH-B2[b,c]	M, H, L, and E (G)
		LDH-C[b,c]	E (G)
Malate dehydrogenase	1.1.1.37	*sMDH-A1*[b,c]	L (E), H (D)
		sMDH-A2[b,c]	H (D)
		sMDH-B1,2[b,c]	H (D), M and L (E)
Malic enzyme ($NADP^+$)	1.1.1.40	*mMEP-2*[b,c]	M (I)
		sMEP-1[b,c]	M (I)
Mannose-6-phosphate isomerase	5.3.1.8	*MPI*[b,c]	H (C, H)
α-Mannosidase	3.2.1.24	*αMAN*[c]	H (I)
Peptidase	3.4.-.-		
Glycyl-leucine activity		*PEPA** (*GL*)[c]	H (G)
Leucyl-glycyl-glycine activity		*PEPB-1** (*LGG*)[b,c]	E (F, J)
Phosphoglucomutase	5.4.2.2	*PGM-1*[b,c]	H (D)
		PGM-2[b,c]	H (D), M (H)
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH*[b,c]	M (A), L (B)
Triose-phosphate isomerase	5.3.1.1	*TPI-1*[b,c]	E (G), H (J)
		TPI-2[c]	E (G), H (J)
		TPI-3[b,c]	E (G), H (J)
		TPI-4[b,c]	E (G), H (J)

[a]A and B, CAME7.2 and CAME7.4, respectively (Aebersold et al. 1987); C, D, E, and F, modified Clayton and Tretiak (1972) to pH 7, 6.5, 6.1, and 5.75, respectively; G, R (Ridgway et al. 1970); H, MF (Markert and Faulhaber 1965); I, TC4 (buffer *a* of Schaal and Anderson 1974); J, TG (0.04 M Tris, 0.12 M glycine, equal concentration for gel and electrode).

[b]Locus included in principal component and multiple analysis of variance analyses.

[c]Locus included in gene diversity analysis and construction of neighbor-joining tree.

tissues from approximately 100 fish per location were stored on packs of frozen gel refrigerant and frozen at −20°C within hours of collection, then shipped to the Auke Bay Laboratory and stored as soon as possible at −80°C. Protein electrophoresis was conducted as described by Aebersold et al. (1987). Specific enzymes were stained according to Harris and Hopkinson (1976) or Aebersold et al. (1987). Loci for which data were obtained, the tissues in which they were expressed, and the buffer systems with which they were resolved are listed in Table 2.

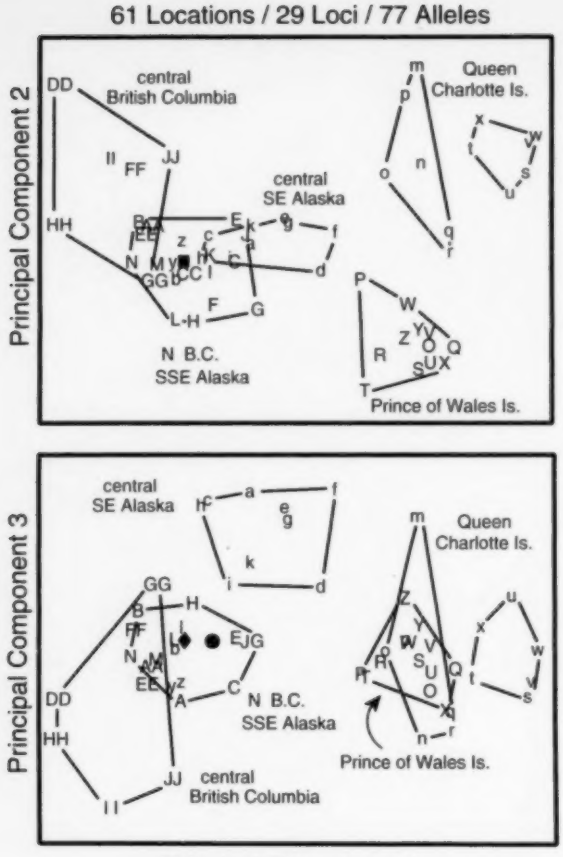

FIG. 2. Principal component analysis of southeast Alaska and northern British Columbia chum salmon for allelic frequencies of 29 loci (see Table 2). Letters correspond to location names in Table 1 and Figs. 1 and 3. N BC, = northern British Columbia; SSE Alaska, southern southeast Alaska. Letters that are indistinguishable as a result of overlap are represented by symbols: ■, A, D, I, DD; ◆, D, BB, CC; ●, F, I, K.

Departure from Hardy–Weinberg expectations was examined with χ^2 goodness-of-fit tests. Because Pearson's goodness-of-fit test is not asymptotic to the χ^2 distribution at low expected frequencies, a Monte Carlo resampling program was used to generate distributions of X^2 statistics for each test (A.J. Gharrett, unpublished data).

Hardy–Weinberg expectations were not tested in two situations: (*i*) locus *PGM-1** and (*ii*) isoloci. *PGM-1** has a null allele (nonstaining); only four of the possible six phenotypes were detectable for the three alleles we observed. Therefore, conformance to Hardy–Weinberg frequencies was not tested because genotypes could not be inferred. Other statistical tests using data from this locus used the observed phenotypic frequencies; however, assumed genotypic frequencies were used to calculate genetic distance and gene diversity. Hardy–Weinberg expectations were also not tested for comigrating, duplicated loci (isoloci) (Allendorf and Thorgaard 1984). Variation at isoloci was assigned to the loci based on maximum-likelihood methods (Waples 1988). Generally, both loci were assigned the variation when an isolocus was highly variable (*sAAT-1,2**); for isoloci with low variability, only one locus was assigned the variation, and the other was treated as monomorphic (*sMDH-B1,2**, *GPI-B1,2**, *mAH-1,2**).

Homogeneity of samples among years within locations was tested with log-likelihood ratio analysis (*G*-tests) (Sokal and Rohlf 1981). Homogeneity among locations and groups of locations was tested after initial descriptive analyses had been made. Because the *G*-statistic is not necessarily asymptotic to the χ^2 distribution when low-frequency alleles are involved, we generated a distribution of *G*-statistics for each test, using a Monte Carlo process. The *G*-statistic distributions were made by reconstructing each set of allele frequencies for a locus by randomly drawing from the weighted average of the allele frequencies (with replacement) the number of alleles originally observed in each collection and computing a *G*-statistic. The process was repeated 2000 times to generate a distribution. The proportion of *G*-statistics generated by the Monte Carlo process that exceeded the observed *G*-statistic was inferred as the probability of the test.

Relationships among collections were examined using two descriptive methods: principal component analysis (SAS Institute Inc. 1988) and construction of an unrooted tree using genetic distance. Principal component analysis was computed with the arc sine – square root transformed frequencies of $N - 1$ alleles. Alleles expressed in fewer than five fish were combined with the allele that had the most similar mobility. A neighbor-joining tree (Saitou and Nei 1987) was constructed with the chord distances (Cavalli-Sforza and Edwards 1967) between each pair of populations.

Multivariate analysis of variance (MANOVA) of arc sine – square root transformed allelic frequencies was used to examine the significance of the geographic structure of the populations sampled. Significance was inferred using Wilks' λ. Alleles expressed in fewer than five fish were combined with the allele that had the most similar mobility. Concern that low levels of variation at some loci might produce spurious results led us to determine significance levels by using a Monte Carlo resampling program to generate a distribution of *F*-statistics as described for the distribution of *G*-statistics (A.J. Gharrett, unpublished data). The proportion of *F*-statistics generated by the resampling process that exceeded the observed *F*-statistic generally was similar to the probability obtained from the *F*-distribution.

Genetic diversity was partitioned into components attributable to the average amount of variation at a collection site (H_S/H_T), differences among collections within a region [$G_{SR} = (H_R - H_S)/H_T$], and differences among regions [$G_{RT} = (H_T - H_R)/H_T$] (Chakraborty and Leimar 1987). H_S is the average expected heterozygosity of each sample site, H_R is the average expected heterozygosity within each region, and H_T is the overall average expected heterozygosity. In this analysis, average allelic frequencies were calculated from unweighted averages of the collections.

Results

Forty-six loci were resolved from 21 enzyme-specific stains. Of these, 42 were variable (including four isoloci), and 4 loci were monomorphic (α*MAN**, *mIDHP-2**, *LDH-B1**, and *IDDH-1**). Allelic frequencies are listed in Appendix 1.

TABLE 3. Relative rank of the 12 most important loci with common allele frequencies <0.95 in the first three factors of the principal component analysis. *P* values are also included for the MANOVA and log-likelihood ratio (*G*-test) analyses.

Locus	PC1	PC2	PC3	MANOVA	*G*-test
*sAAT-3**	1	11	10	3×10^{-11}	$<10^{-6}$
*mIDHP-1**	2	9	6	2×10^{-11}	$<10^{-6}$
*mAAT-1**	3	12	9	5×10^{-10}	$<10^{-6}$
*PGM-1**	4	8	12	4×10^{-7}	$<10^{-6}$
*ESTD-1**	5	3	11	3×10^{-10}	$<10^{-6}$
*sMDH-A1**	6	6	8	8×10^{-9}	$<10^{-6}$
*G3PDH-2**	10	1	7	4×10^{-5}	$<10^{-6}$
*sAAT-1,2**	12	2	1	1×10^{-6}	$<10^{-6}$
*PEPB-1**	7	4	5	1×10^{-12}	$<10^{-6}$
*MPI**	11	5	2	6×10^{-4}	$<10^{-6}$
*mMEP-2**	9	7	3	2×10^{-6}	$<10^{-6}$
*FH**	8	10	4	1×10^{-2}	$<10^{-6}$

Departure from Hardy–Weinberg expectations occurred in 34 of a possible 1406 tests, which is less than expected by chance alone at an $\alpha = 0.05$. These departures occurred in fewer than half the loci analyzed; 8 of the 34 significant values occurred at the *G3PDH-2** locus and were due to an excess of homozygotes. The remaining significant departures from Hardy–Weinberg equilibrium were spread among those loci in which departures occurred.

At 15 locations, samples were collected in more than 1 yr. Although tests at individual loci showed some heterogeneity (26 of 349 tests), after adjustment for multiple testing (Cooper 1968), they were not significant. The overall tests (over all loci) were significant for 3 of the 15 locations (Herman Creek, Old Tom Creek, and Cruz Cove); but after taking multiple testing into account, only Herman Creek was heterogeneous over years. Because the samples were of consistently good quality and were collected from the same location at the same time of year, we pooled the allelic frequencies of collections within a location, including the one with a significant overall *G*-statistic, for subsequent analyses.

We observed distinct clustering of collections with principal component analysis that corresponded to six geographic regions (Fig. 2). This analysis was based on 29 variable loci (see Table 2) common to all collections, with a total of 77 alleles. The first three principal components accounted for 21, 11, and 8% of the observed genetic variation, respectively. The first component was useful in separating the Queen Charlotte Islands and Prince of Wales Island collections from the other collections. Collections in the Queen Charlotte Islands region separated into two groups: one included all the collections from the east side of Moresby Island (the more southerly of the two largest Queen Charlotte Islands; collections *s–x*); the second group consisted of collections from the outer coast, the north end of Graham Island (the more northerly large island), and the channel between Moresby and Graham islands (collections *m–r*). The second principal component was useful in separating the Queen Charlotte Islands collections from the Prince of Wales Island collections, and the third component splits the central southeast Alaska collections from southern southeast Alaska and north-central British Columbia collections.

The principal component plots suggest some separation between southern southeast Alaska – northern British Columbia (Portland and Observatory Inlets) collections and the collections from north to central British Columbia (DD–JJ); however, the differences are less distinct than between the other groups because several collections overlap. The two northern southeast Alaska collections did not form a distinct group. The Lynn Brothers collection (*k*) clustered with the central southeast Alaska collections, and the collection from Herman Creek (*l*), a Chilkat River tributary, clustered with southern southeast Alaska – northern British Columbia collections. The most variable loci, with a common allele frequency <0.95, useful in distinguishing geographic regional differences, are listed in Table 3. Some of the less common alleles, from loci with more than two alleles, were also important contributors (*sMDH-B1,2*72*, *sMDH-B1,2*50*, *sMDH-B1,2*129*; *MPI*e*[1]; *PEPB-1*b*, *PEPB-1*c*, *PEPB-1*d*, *PEPB-1*e*[2]; *GPI-A*95*, *GPI-A*105*).

Genetic distances used to construct a neighbor-joining tree were calculated from 40 loci (see Table 2), including invariant loci, that were common to all collections. The unrooted tree revealed virtually identical geographic groupings of the collections (Fig. 3). The one exception, the Port Real Marina collection (X), clustered with a Queen Charlotte Islands branch.

We grouped the collections for MANOVA based on these descriptive analyses. Testing for geographic structure used the six geographical regions as a fixed effect on the transformed allelic frequencies for a total of 77 alleles at 29 loci (see Table 2). The test was highly significant (Wilks' $\lambda = 1 \times 10^{-8}$, $F_{240,45} = 7.2093$, $P = 4 \times 10^{-12}$). All but seven loci (*PGM-2**, *CK-B**, *IDDH-2**, *sMEP-1**, *GPI-B1,2**, *TPI-3**, and *TPI-4**) had significant univariate *F*-tests based on the bootstrap resampling analysis. Allelic frequency data for *sIDHP-2**, *mAH-3**, *mAH-1,2**, and *sAH** were unavailable for Mussel River, Nekite Channel, Nekite River, Klownik Creek, Kitimat River, Ecstall River, Pallant Creek, Tombstone River, Marten River, Klahini River, Traitor's Creek, Eulachon Creek, Tokeen Creek, Elena Creek, Eliza Creek, and Gambier Creek. When these collections were removed from the analysis, the univariate *F*-statistics and *G*-tests from the log-likelihood analysis were also significant for *sIDHP-2**, *mAH-3**, and *mAH-1,2**.

The hierarchical log-likelihood ratio analysis corroborated the MANOVA results (approximate *F*-statistic = 11.325, 235 df in the numerator, 2585 df in the denominator, $P < 1 \times 10^{-6}$), where the *F*-statistic is approximated by $(G_{between}/df_{between})/(G_{within}/df_{within})$ (Appendix 2). We also found significant heterogeneity within every region, except the collections from the eastern side of Moresby Island (collections *s–x*). This suggests that additional genetic structure is present within the regional areas.

For the gene diversity analysis, we used the six geographic regions observed in the principal component analysis and neighbor-joining tree to estimate the proportion of variation attributable to three levels of hierarchy: within collections (H_S/H_T), among collections within regions (G_{SR}), and among regions (G_{RT}). Most of the genetic diversity (97% on average) was found within sampling locations; the

[1]Two buffers are required to resolve this locus; the *e* allele has mobilities 86 and 94 on CA7 and MF gels, respectively.

[2]Two buffers are required to resolve this locus; the mobilities for the *a*, *b*, *c*, *d*, and *e* alleles are *−100 and *100, *−126 and *100, *−127 and *59, *−146 and *59, and *−72 and *110 at CA5.75 and TG or R, respectively.

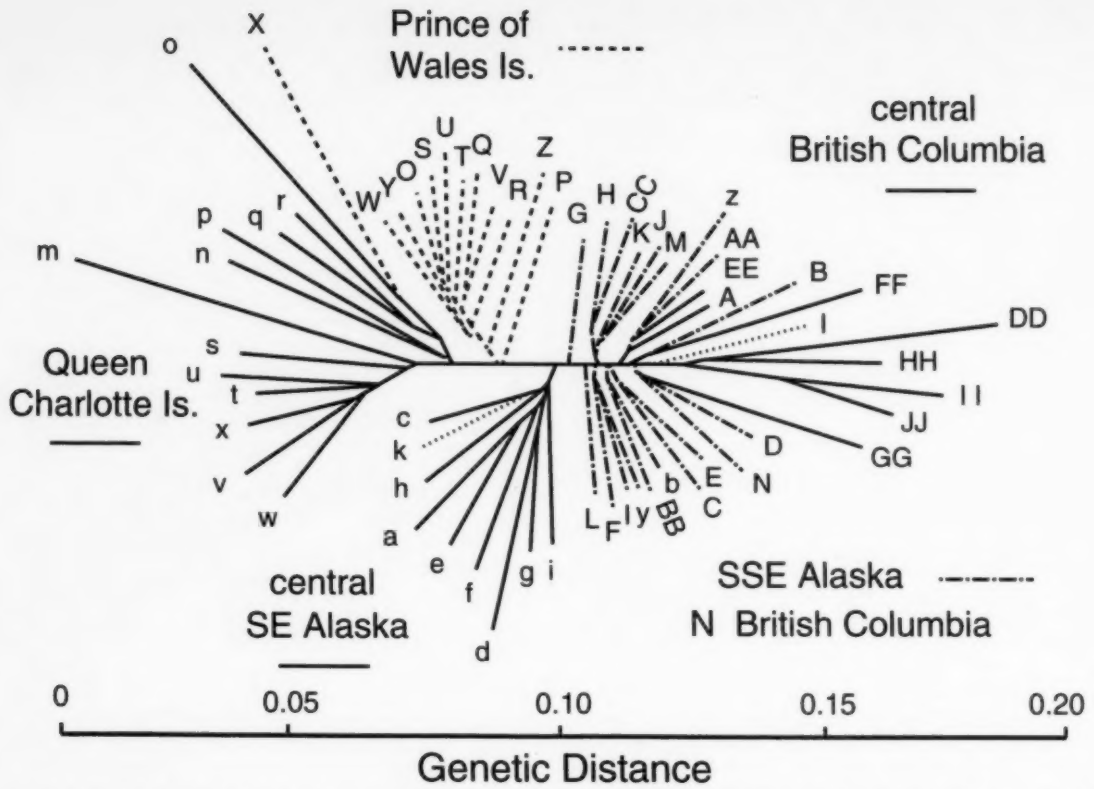

FIG. 3. Neighbor-joining tree (Saitou and Nei 1987) using genetic distances (Cavalli-Sforza and Edwards 1967) estimated from allelic frequencies of 40 loci (see Table 2). Letters correspond to location names in Table 1 and Figures 1 and 2. Genetic distances for populations "*k*" and "*l*" are indicated as (---) in the central southeast Alaska and central British Columbia population groups, respectively.

remaining variability was found almost equally within regions (1.3%) and among regions (1.4%).

Discussion

We observed significant allelic frequency differences both within and among regions; however, variation among regions was significantly greater. Data for almost half of the most important loci useful in distinguishing geographic regions in this study have not been previously reported (Okazaki 1982; Beacham et al. 1987). All but one location (Herman Creek) sampled in more than 1 yr had no significant year-to-year allelic frequency differences, indicating substantial stability of genetic structure within locations. Heterogeneity in Herman Creek samples suggests that subpopulation structure may exist in this location, a small tributary of the Chilkat River.

We compared allele frequencies at six loci (*sIDHP-2**, *mIDHP-1**, *mMEP-2**, *PGDH**, *G3PDH-2**, and *PEPB-1**) with those previously reported by Beacham et al. (1987) for 19 locations in northern British Columbia common to both studies. Of a possible 108 *G*-tests, 11 were significant. *PEPB-1** was the only locus that differed consistently among data sets; 4 of the 11 significant comparisons were due to this locus, which was more variable in our data than in Beacham et al. (1987). On closer examination, the two data sets were more similar than the initial analysis indicated. Beacham et al. (1987) had data for more than 1 yr for many locations, and we found that our data resembled theirs for at least 1 yr in 7 of the 11 significant tests. Thus, some of the differences may have been due as much to differences among years within the Beacham et al. (1987) study as to differences between their and our data sets.

Our analysis indicated the existence of six regional groups: Prince of Wales Island area, central southeast Alaska, southern southeast Alaska – northern British Columbia, north-central British Columbia, and two groups in the Queen Charlotte Islands area. Allele frequencies of mainland populations differed from those of the island populations; perhaps the mainland was colonized by fish moving north or south, rather than east from the two island groups. The Queen Charlotte Islands and Prince of Wales Island groups were clearly different from the other populations.

The Queen Charlotte Islands area had two distinct groups of populations. The greatest amount of heterogeneity observed within a region occurred in the northern and western Queen Charlotte Islands. Recent geologic work in the Queen Charlotte Islands has revealed that parts of this area were almost certainly ice free during the height of the last glaciation (Warner et al. 1982). Perhaps several chum salmon populations survived here, and due to isolation over time, have been subject to genetic drift longer than other populations examined in this

study. The large amount of heterogeneity suggests a strong affinity for these populations to home to their natal streams. Alternatively, the differences reflect different local selection regimes or a combination of local selection and genetic drift.

A different situation exists on the eastern side of the Queen Charlotte Islands (collections *s–x*). These populations have similar allelic frequencies, which suggests that they may have descended from the same gene pool in more recent times. During the last glaciation, the shoreline here was lower because of lower ocean levels, and the configuration of the eastern side of Graham Island would have been greatly different from today. The present shallow passage between the northern half of the Queen Charlotte Islands and the mainland would have been land or ice, which would have blocked fish movement along the eastern side of the islands. The chum salmon on the inner coast probably survived as a smaller group, limited to the southeastern end of Moresby Island, and may have repopulated streams on the northeastern side of the Queen Charlotte Islands when sea levels rose as the glaciers retreated. Additional samples from the Queen Charlotte Islands, particularly the outer coast, may provide greater resolution of population structure relative to past glaciation and additional information about the size and nature of the refugium.

The Prince of Wales Island populations also exhibit heterogeneity, but less than the Queen Charlotte Islands populations. Information about past glaciation in this area is limited, but local areas of the outer coast of southeast Alaska may have been ice free during the late Wisconsin (Mann 1986; J. Baichtal, U.S. Forest Service, Ketchikan, AK 99901, personal communication). Perhaps part of the Prince of Wales Island area also served as a separate refuge during the height of this period. This could explain the large difference between these two regions. The Prince of Wales Island area was covered with ice more recently than the Queen Charlotte Islands area; repopulation of this region in more recent times would account for its greater homogeneity than the Queen Charlotte Islands area. Future study of northern southeast Alaska populations, particularly western Baranof Island, may provide further clues about the postglacial dispersal and possible refugia patterns along this coast.

Allozyme data are now available for much of the geographic distribution of chum salmon. Analysis of this baseline may reveal how postglacial dispersal resulted in the present distribution. Chum salmon probably survived in more than one unglaciated area (McPhail and Lindsey 1970), but only now is information available that allows us to infer patterns of colonization. It will be interesting to see whether the allele frequencies of chum salmon populations in southeast Alaska and northern British Columbia are more similar to those of populations from the Bering refuge (e.g., Yukon River, either via the marine environment around the Alaska Peninsula and Gulf of Alaska, or reverse flow of the Yukon River headwaters into southeast Alaska) or to those of populations from the Pacific refuge in Washington and Oregon.

The geographic basis for heterogeneity among regions is confounded in part by spawning-time differences. For example, the Prince of Wales Island and Queen Charlotte Islands chum salmon populations spawn in the fall, whereas the mainland populations of both southeast Alaska and northern British Columbia spawn mainly in the summer (Pacific Salmon Commission 1987). The summer runs are more protracted, and their timing may overlap slightly with that of the fall runs. Also, more southerly populations tend to spawn later.

Although the gene diversity values reported here cannot be compared directly with those of other studies because the loci included in different studies are not the same, the values for chum salmon populations from central southeast Alaska to central British Columbia are similar to those reported elsewhere (Okazaki 1982; Beacham et al. 1985, 1987; Wilmot et al. 1994).

National and international interest exists for using genetic information to estimate stock contributions in mixed-stock fisheries. Our genetic data can now be used in simulations to determine how accurately and precisely the allelic frequency differences can distinguish stock composition in a mixture. If our data can be used to estimate stock composition successfully, as has been done for chum fisheries in southern British Columbia and Washington (Beacham et al. 1987; S. Phelps, Washington Department of Fisheries, P.O. Box 43151, Olympia, WA 98504, personal communication; L. Hop Wo, Canada Department of Fisheries and Oceans, 3225 Stephenson Point Road, Nanaimo, BC, V9T 1K3, personal communication) it should be useful for management of mixed-stock chum salmon fisheries within Southeast Alaska and those fisheries near the southeast Alaska – British Columbia border.

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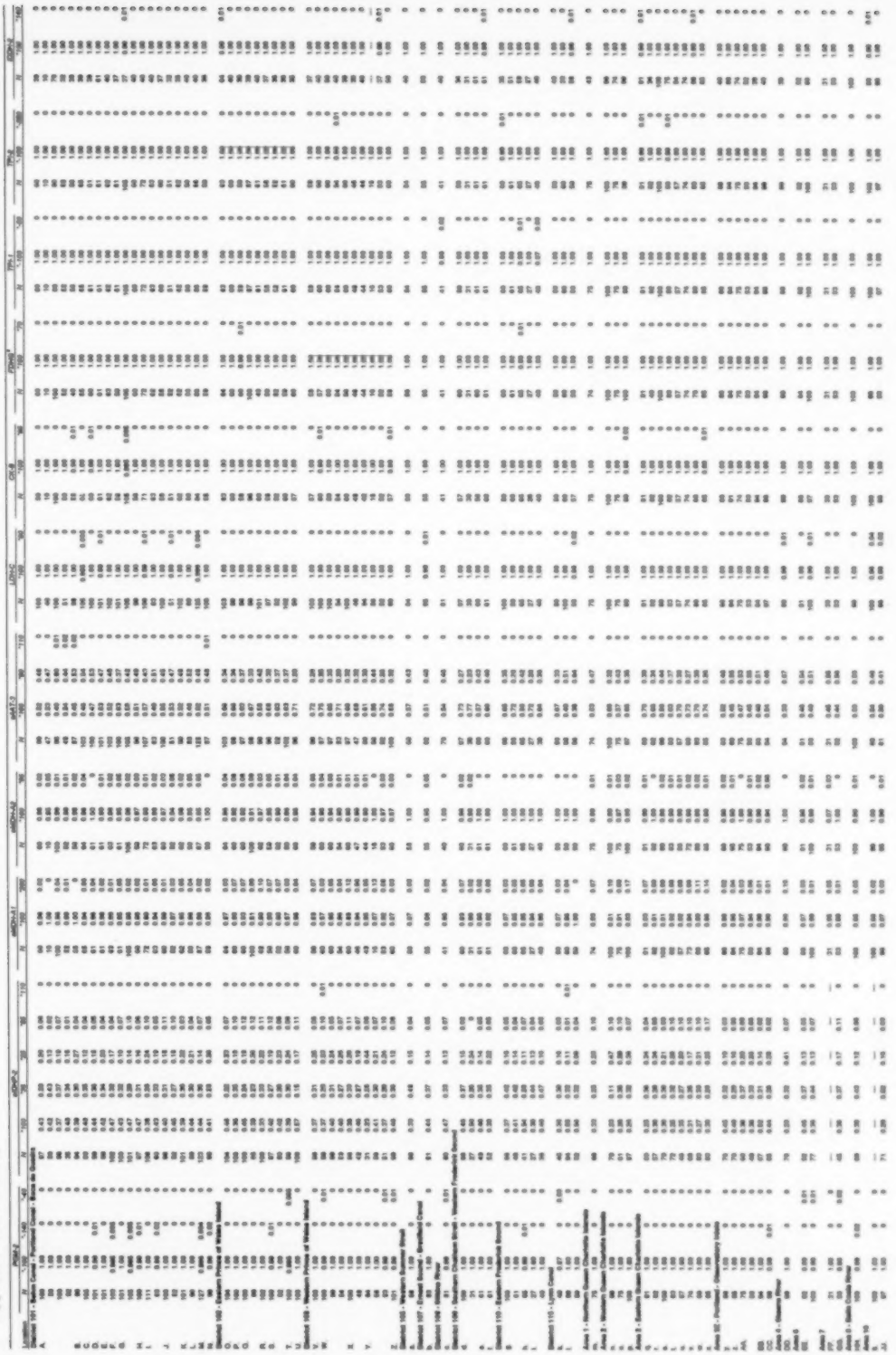
Appendix 1. Allozyme variation in collections of chum salmon from southeast Alaska and northern British Columbia. *N* = number of fish successfully screened at each locus. Alleles are designated by their mobility relative to the most common allele (**100*) and as described in Shaklee et al. (1990). Monomorphic loci include α*MAN**, *mIDHP-2**, *LDH-B1**, and *IDDH-1**. Rare variation is footnoted. Dashes (---) indicate no data.

Location		sAAT-1,2 N	*100	*120	*80	*68	mAAT-1 N	*100	*120	*70	ALAT N	*100	*80	*108	EST-1 N	*100	*91	*110	*80	mIDHP-1[1] N	*100	*80	LDH-A1[2] N	*100	*80	*variable	LDH-B2 N	*100	*120	PEP-1 (LGD)[3] N	*a	*b	*c	*d	*e
District 101 - Behm Canal - Portland Canal - Boca de Quadra																																		
A. Fish	1988	100	0.84	0.16	0	0	100	0.71	0.23	0.07	99	0.75	0.25	0	100	0.95	0.05	0	0	100	0.87	0.13	99	0.96	0.04	0	100	1.00	0	60	0.77	0.03	0.07	0.12	0
	1987	50	0.87	0.13	0	0	99	0.69	0.26	0.05	50	0.81	0.18	0.01	50	0.96	0.04	0	0	50	0.89	0.16	50	0.97	0.03	0	50	1.00	0	10	0.80	0	0	0.20	0
early	1988	100	0.87	0.13	0	0	99	0.65	0.29	0.07	100	0.75	0.23	0.01	100	0.97	0.03	0	0	100	0.90	0.10	100	0.97	0.03	0	100	0.99	0.01	60	0.75	0.05	0.04	0.16	0
late	1988	50	0.90	0.10	0	0	52	0.82	0.34	0.06	49	0.84	0.16	0	52	0.94	0.06	0	0	52	0.85	0.15	52	0.97	0.03	0	50	1.00	0	50	0.79	0.03	0.04	0.14	0
B. Tombstone		96	0.89	0.11	0	0	99	0.85	0.30	0.05	96	0.83	0.17	0	97	0.94	0.06	0	0	97	0.90	0.10	98	0.98	0.02	0	100	1.00	0	65	0.81	0.03	0.05	0.13	0
C. Marten		104	0.87	0.13	0	0	104	0.75	0.18	0.07	93	0.80	0.19	0.01	104	0.93	0.07	0	0	105	0.95	0.05	105	0.98	0.02	0	101	0.99	0.01	98	0.75	0.09	0.05	0.10	0
D. Kete		101	0.91	0.09	0	0	98	0.74	0.20	0.06	101	0.79	0.20	0.01	101	0.93	0.07	0	0	101	0.90	0.10	101	0.99	0.01	0	101	0.99	0.01	98	0.80	0.03	0.07	0.10	0
E. Blossom		99	0.90	0.10	0	0	100	0.72	0.23	0.05	91	0.83	0.17	0	101	0.93	0.07	0.01	0	100	0.93	0.07	100	0.97	0.03	0	103	0.995	0.005	65	0.80	0.07	0.03	0.10	0
F. Wilson		102	0.86	0.14	0	0	102	0.69	0.27	0.05	92	0.82	0.15	0.03	103	0.93	0.07	0	0	102	0.90	0.10	102	0.97	0.03	0	101	0.99	0.01	61	0.75	0.05	0.02	0.18	0
G. Trefon's	1986	101	0.88	0.12	0	0	101	0.77	0.18	0.04	91	0.79	0.21	0	101	0.91	0.09	0	0	100	0.89	0.11	101	0.97	0.03	0	101	1.00	0	105	0.74	0.05	0.01	0.20	0
	1988	104	0.88	0.12	0	0	105	0.73	0.21	0.05	103	0.77	0.22	0.005	105	0.95	0.05	0	0	105	0.89	0.11	105	0.96	0.02	0	105	1.00	0	105	0.74	0.05	0.01	0.20	0
H. Carroll		100	0.88	0.12	0	0	99	0.62	0.36	0.03	99	0.69	0.31	0	100	0.94	0.06	0	0	100	0.92	0.08	100	0.95	0.05	0	100	0.99	0.01	90	0.82	0.03	0.04	0.12	0
I. Portage	1988	108	0.90	0.10	0	0	110	0.80	0.20	0.04	111	0.86	0.13	0.01	111	0.96	0.04	0	0	110	0.93	0.07	111	0.98	0.02	0	111	0.99	0.01	72	0.83	0.02	0.03	0.09	0
	1988	89	0.89	0.11	0	0	93	0.83	0.29	0.07	93	0.80	0.19	0.01	93	0.94	0.06	0	0	93	0.90	0.10	93	0.99	0.01	0	93	1.00	0	93	0.80	0.03	0.03	0.13	0
J. King	1986	100	0.86	0.14	0	0	100	0.73	0.22	0.05	90	0.75	0.24	0.01	100	0.93	0.07	0	0	98	0.92	0.08	100	0.95	0.05	0	100	1.00	0	93	0.73	0.04	0.04	0.10	0
	1988	52	0.88	0.11	0	0	52	0.74	0.21	0.05	47	0.78	0.20	0.02	52	0.93	0.07	0	0	52	0.94	0.06	52	0.96	0.04	0	52	1.00	0	52	0.87	0	0.02	0.12	0
K. Kishmi		101	0.88	0.12	0	0	102	0.69	0.29	0.02	100	0.75	0.23	0.01	102	0.93	0.07	0	0	102	0.93	0.07	102	0.97	0.03	0	102	1.00	0	52	0.81	0.05	0.03	0.10	0
L. Eulachon		87	0.88	0.12	0	0	89	0.72	0.23	0.05	80	0.76	0.21	0.01	90	0.96	0.05	0	0	90	0.87	0.13	90	0.99	0.01	0	90	1.00	0	53	0.80	0.03	0.04	0.13	0
M. Grant		127	0.90	0.10	0	0	126	0.69	0.27	0.04	125	0.79	0.22	0	126	0.96	0.04	0	0	127	0.92	0.07	127	0.98	0.02	0	127	1.00	0	97	0.81	0.03	0.03	0.11	0
N. Harmon		100	0.85	0.15	0	0	99	0.71	0.27	0.02	100	0.87	0.13	0	99	0.95	0.05	0.01	0	100	0.89	0.11	100	0.97	0.03	0	100	1.00	0	99	0.81	0.02	0.06	0.11	0
District 102 - Eastern Prince of Wales Island																																		
O. Kugal		104	0.85	0.15	0	0	104	0.86	0.11	0.03	102	0.88	0.12	0	104	0.95	0.14	0	0	103	0.97	0.03	103	0.99	0.02	0	104	0.99	0.02	64	0.74	0.03	0.02	0.20	0
P. Aiken		100	0.87	0.13	0	0	100	0.91	0.09	0.01	99	0.86	0.15	0	100	0.93	0.07	0	0	100	0.93	0.07	100	0.99	0.01	0	100	1.00	0	98	0.78	0.03	0.03	0.17	0
Q. Disappearance	1986	100	0.85	0.15	0	0	100	0.85	0.11	0.03	99	0.83	0.17	0	100	0.87	0.13	0	0	99	0.99	0.01	99	0.94	0.06	0	100	0.99	0.01	97	0.76	0.03	0.05	0.16	0
	1988	98	0.86	0.14	0	0	100	0.86	0.13	0.02	98	0.90	0.20	0	100	0.93	0.18	0	0	103	0.97	0.03	98	0.97	0.03	0	100	0.99	0.01	97	0.75	0.03	0.03	0.19	0
R. Lagoon		101	0.89	0.11	0	0	102	0.83	0.13	0.04	99	0.75	0.22	0	102	0.97	0.13	0	0	98	0.96	0.04	98	0.98	0.04	0	101	0.99	0.01	98	0.72	0.06	0.04	0.18	0
S. Old Tom	1986	100	0.86	0.14	0.000	0	100	0.89	0.07	0.05	97	0.80	0.20	0	100	0.78	0.22	0	0	100	0.96	0.04	100	1.00	0	0	100	0.99	0.01	59	0.72	0.04	0.05	0.19	0
	1988	52	0.90	0.10	0	0	50	0.89	0.10	0.02	47	0.81	0.19	0	52	0.90	0.10	0	0	52	1.00	0	51	0.98	0.02	0	52	0.97	0.03	52	0.73	0.08	0.05	0.15	0
T. Cabin		100	0.85	0.15	0	0	103	0.89	0.08	0.04	92	0.86	0.14	0	103	0.86	0.14	0	0	103	0.98	0.07	103	0.96	0.04	0	103	0.98	0.02	62	0.77	0.04	0.04	0.14	0
U. Karta		100	0.87	0.13	0	0	99	0.86	0.06	0.09	97	0.75	0.25	0	100	0.87	0.13	0	0	99	0.97	0.03	100	0.98	0.02	0	100	0.99	0.01	60	0.74	0.04	0.02	0.20	0
District 103 - Western Prince of Wales Island																																		
V. Coco Harbor		99	0.87	0.13	0	0	100	0.92	0.15	0.03	100	0.84	0.17	0	100	0.87	0.13	0	0	100	0.95	0.05	100	0.97	0.03	0	100	0.99	0.01	59	0.76	0.03	0.03	0.17	0
W. Brent's Bay	1986	100	0.90	0.10	0	0	100	0.89	0.11	0.01	99	0.92	0.18	0	99	0.90	0.09	0	0.01	100	0.97	0.03	100	0.95	0.05	0	100	0.99	0.01	59	0.78	0.03	0.03	0.18	0
	1987	100	0.85	0.14	0	0	100	0.89	0.11	0.03	99	0.83	0.17	0	100	0.89	0.10	0	0.01	94	0.94	0.06	94	0.96	0.04	0	94	0.95	0.04	54	0.77	0.01	0.08	0.18	0
	1988	94	0.83	0.17	0	0	94	0.89	0.10	0.02	94	0.91	0.09	0	94	0.83	0.17	0	0	100	0.91	0.09	100	0.97	0.03	0	100	0.96	0.02	60	0.72	0.07	0.07	0.14	0
X. Port Real Marina	1986	100	0.86	0.14	0	0	100	0.87	0.12	0.01	99	0.86	0.15	0	100	0.83	0.17	0	0	100	0.91	0.09	100	0.98	0.02	0	100	0.99	0.01	59	0.75	0.05	0.04	0.16	0
	1988	48	0.83	0.17	0	0	48	0.84	0.16	0	48	0.78	0.22	0	48	0.86	0.15	0	0	47	0.91	0.09	48	0.99	0.02	0	48	0.99	0.01	44	0.67	0.03	0.11	0.19	0
Y. Crut Cove	1986	94	0.85	0.15	0	0	94	0.89	0.10	0.02	---	---	---	---	94	0.85	0.14	0	0	93	0.95	0.05	93	0.96	0.04	0	93	0.95	0.05	10	0.84	0.03	0.03	0.09	0
	1987	99	0.92	0.08	0	0	99	0.80	0.19	0.01	94	0.79	0.21	0	99	0.95	0.05	0	0	93	0.95	0.05	93	0.98	0.02	0	99	0.95	0.05	53	0.77	0.02	0.05	0.16	0
	1988	53	0.83	0.11	0	0	53	0.88	0.09	0.03	52	0.79	0.18	0.03	53	0.77	0.23	0	0	53	0.95	0.05	53	0.98	0.02	0	53	0.96	0.04	53	0.79	0.08	0	0.16	0
Z. Tokeen		101	0.89	0.11	0	0	101	0.74	0.23	0.03	99	0.84	0.16	0	100	0.79	0.22	0	0	101	0.99	0.02	100	0.96	0.04	0	101	0.99	0.01	99	0.79	0.09	0	0.16	0
District 105 - Western Dixon Strait																																		
a. Irish		97	0.92	0.08	0	0	99	0.79	0.18	0.04	99	0.90	0.10	0	98	0.97	0.03	0	0	98	0.94	0.06	96	0.97	0.03	0	99	0.99	0.01	99	0.85	0.04	0	0.10	0
District 107 - Ernest Sound - Bradfield Canal																																		
b. Harding		94	0.86	0.12	0	0	94	0.85	0.20	0.07	91	0.81	0.19	0.01	95	0.93	0.07	0	0	94	0.93	0.07	94	0.97	0.03	0	95	0.99	0.01	95	0.75	0.02	0.08	0.14	0
District 108 - Stikine River																																		
c. North Arm		81	0.92	0.08	0	0	80	0.88	0.20	0.06	81	0.80	0.20	0	81	0.98	0.04	0	0	81	0.92	0.08	81	0.95	0.04	0	81	1.00	0	41	0.80	0.02	0.04	0.13	0
District 109 - Southern Chatham Strait - Western Frederick Sound																																		
d. Lever's Cove	1987	100	0.90	0.06	0	0.04	100	0.79	0.21	0.01	100	0.91	0.19	0	100	0.93	0.07	0	0	100	0.95	0.05	100	0.97	0.03	0	100	0.99	0.01	60	0.87	0.03	0.05	0.04	0
	1990	31	0.91	0.06	0	0.02	31	0.76	0.24	0	27	0.87	0.13	0	31	0.92	0.08	0	0	30	0.98	0.02	31	0.98	0.02	0	31	1.00	0	30	0.87	0.07	0.05	0.02	0
e. Eliano Bay		61	0.95	0.05	0	0	61	0.77	0.20	0.02	61	0.93	0.07	0.01	61	0.95	0.05	0	0	61	0.93	0.07	61	0.94	0.06	0	61	1.00	0	61	0.82	0.03	0.04	0.11	0
f. Elbo Harbor		91	0.96	0.02	0	0	91	0.74	0.20	0.07	90	0.92	0.18	0	91	0.89	0.11	0	0	91	0.93	0.07	91	0.95	0.05	0	91	0.99	0.01	61	0.75	0.05	0.05	0.14	0
District 110 - Eastern Frederick Sound																																		
g. Donkey	1986	60	0.91	0.87	0	0.02	99	0.70	0.27	0.04	99	0.83	0.17	0	100	0.97	0.03	0	0	100	0.95	0.05	99	0.94	0.06	0	100	0.99	0.01	60	0.82	0.01	0.03	0.14	0
	1988	61	0.91	0.05	0	0.01	99	0.80	0.17	0.03	98	0.82	0.20	0	61	0.95	0.05	0	0.01	60	0.93	0.07	61	0.98	0.04	0	61	0.98	0.02	61	0.81	0.02	0.03	0.13	0
h. Pybus Bay		95	0.92	0.08	0.004	0	65	0.73	0.25	0.02	65	0.92	0.18	0	65	0.95	0.05	0	0	65	0.98	0.04	65	0.95	0.04	0	65	1.00	0	65	0.84	0.01	0.03	0.10	0
i. Gambier Bay	1988	27	0.93	0.10	0	0	27	0.63	0.31	0.05	34	0.87	0.13	0	27	0.94	0.06	0	0	27	0.96	0.04	27	0.98	0.02	0	27	1.00	0	27	0.80	0.02	0.02	0.17	0
	1990	40	0.91	0.09	0	0	39	0.69	0.28	0.04	39	0.84	0.16	0	40	0.95	0.04	0.01	0	40	0.90	0.10	40	0.97	0.03	0	40	1.00	0	39	0.79	0.09	0.04	0.08	0
District 115 - Lynn Canal																																		
n. Lynn Brothers		50	0.93	0.09	0.01	0	50	0.72	0.24	0.04	49	0.84	0.16	0	50	0.95	0.04	0	0	50	0.92	0.09	50	0.95	0.05	0	50	1.00	0	49	0.80	0.02	0.03	0.15	0
l. Herman	1987	99	0.91	0.09	0	0	99	0.82	0.32	0.05	99	0.87	0.13	0	99	0.96	0.04	0	0	100	0.99	0.11	100	0.96	0.06	0	100	1.00	0	99	0.79	0.09	0.05	0.17	0
	1988	99	0.89	0.11	0	0	99	0.83	0.29	0.09	99	0.89	0.11	0	99	0.92	0.08	0	0	99	0.94	0.06	99	0.98	0.02	0	99	1.00	0	99	0.85	0.02	0.01	0.12	0
Area 1 - Northern Queen Charlotte Islands																																		
m. Amon		75	0.97	0.03	0	0	75	0.85	0.05	0	75	0.69	0.31	0	75	0.94	0.06	0	0	75	1.00	0	75	0.99	0.01	0	75	0.89	0.11	74	0.69	0.04	0	0.26	0
Area 2 - Western Queen Charlotte Islands																																		
n. Mecs		99	0.88	0.12	0	0	100	0.95	0.26	0	97	0.93	0.07	0	100	0.95	0.05	0	0	99	0.97	0.03	99	0.97	0.03	0	100	0.99	0.02	100	0.70	0.01	0.01	0.28	0
o. Peel		75	0.93	0.10	0	0	75	0.79	0.24	0	75	0.79	0.21	0.01	75	0.95	0.05	0	0	75	0.97	0.03	75	0.92	0.09	0	75	0.97	0.03	75	0.80	0.04	0.07	0.29	0
p. Tasu		100	0.91	0.09	0	0	100	0.85	0.15	0	99	0.72	0.28	0.02	100	0.97	0.03	0	0	99	0.94	0.06	97	0.97	0.03	0	100	1.00	0	99	0.81	0.06	0.04	0.31	0
Area 3 - Eastern Queen Charlotte Islands																																		
q. Lagins		89	0.85	0.15	0	0	91	0.80	0.19	0.01	91	0.81	0.19	0.01	91	0.89	0.12	0	0	91	0.98	0.02	91	0.98	0.02	0	91	0.99	0.07	91	0.88	0.03	0.05	0.32	0
r. Deena		92	0.84	0.16	0	0	92	0.81	0.19	0	91	0.88	0.12	0	92	0.86	0.14	0	0	92	0.98	0.04	92	0.96	0.04	0	92	0.98	0.12	92	0.73	0.02	0.01	0.24	0
s. Pollard		100	0.91	0.09	0	0	100	0.93	0.15	0.01	100	0.85	0.15	0	100	0.97	0.03	0	0	100	0.97	0.03	100	0.95	0.05	0	103	0.94	0.07	100	0.71	0.06	0.01	0.23	0
t. Lagoon		91	0.92	0.08	0	0	93	0.83	0.17	0	93	0.86	0.14	0.01	93	0.95	0.05	0	0	93	0.98	0.02	93	0.94	0.05	0.01	93	0.98	0.07	93	0.81	0.03	0.01	0.27	0
u. Selmon		57	0.94	0.06	0	0	57	0.89	0.10	0.01	57	0.92	0.14	0.04	57	0.91	0.09	0	0	57	1.00	0	57	0.94	0.05	0.01	57	0.98	0.04	55	0.70	0.04	0	0.26	0
v. Sedgewick		74	0.91	0.09	0	0	74	0.88	0.11	0.05	74	0.88	0.11	0.04	74	0.91	0.09	0	0	74	0.99	0.03	74	0.92	0.08	0	74	0.99	0.07	73	0.83	0.03	0	0.32	0
w. Bag Harbor		89	0.92	0.08	0	0	89	0.85	0.15	0	88	0.86	0.13	0.02	89	0.90	0.10	0	0	89	0.99	0.01	89	0.93	0.07	0	89	0.96	0.05	89	0.89	0.06	0.02	0.02	0.01
x. Sunrise		84	0.94	0.06	0	0	85	0.83	0.17	0	84	0.85	0.14	0.01	85	0.94	0.06	0	0	85	0.99	0.01	85	0.95	0.05	0	85	0.96	0.04	85	0.84	0.06	0.01	0.25	0
Area 32 - Portland - Observatory Inlets																																		
y. Kahoun		99	0.86	0.14	0	0	99	0.89	0.29	0.04	99	0.92	0.15	0.03	99	0.97	0.03	0	0	99	0.95	0.15	99	0.99	0.01	0	99	0.99	0.01	97	0.79	0.05	0.07	0.09	0
z. Kitsault		95	0.84	0.16	0	0	95	0.89	0.33	0.03	95	0.95	0.14	0.01	95	0.94	0.05	0	0	95	0.99	0.12	95	0.96	0.05	0	95	0.96	0.02	95	0.77	0.02	0.04	0.17	0
aa. Skagon	1988	75	0.93	0.17	0	0	75	0.93	0.32	0.05	75	0.80	0.19	0.01	75	0.99	0.02	0	0	75	0.97	0.13	75	0.96	0.01	0	75	0.99	0.01	73	0.79	0.05	0.03	0.10	0
	1989	51	0.96	0.10	0	0	53	0.88	0.24	0.03	53	0.94	0.15	0.01	53	0.95	0.05	0	0	53	0.89	0.11	53	0.94	0.06	0	53	1.00	0	53	0.79	0.07	0.04	0.10	0
bb. Khutzeymateen		94	0.96	0.11	0	0	94	0.85	0.26	0.07	94	0.79	0.21	0.01	94	0.97	0.03	0	0	94	0.97	0.03	94	0.94	0.06	0	94	1.00	0	94	0.73	0.01	0.08	0.18	0
CC. Town		97	0.87	0.13	0	0	99	0.84	0.30	0.03	99	0.71	0.25	0.01	98	0.95	0.04	0	0	99	0.93	0.10	99	0.97	0.03	0	99	0.99	0.01	99	0.79	0.02	0.09	0.15	0
Area 4 - Skeena River																																		
DD. Estelli		99	0.93	0.07	0	0	99	0.61	0.42	0.17	99	0.90	0.10	0.005	99	0.99	0.01	0	0	99	0.97	0.03	99	0.99	0.01	0	99	1.00	0	99	0.74	0.06	0.03	0.12	0
Area 6																																		
EE. Kitimat	1988	91	0.85	0.15	0	0	92	0.85	0.27	0.03	92	0.92	0.18	0	92	0.97	0.03	0	0	92	0.96	0.14	92	0.97	0.03	0	92	1.00	0	89	0.74	0.03	0.04	0.19	0
	1989	99	0.90	0.10	0	0	100	0.89	0.25	0.05	100	0.90	0.19	0.01	100	0.95	0.05	0	0	100	0.97	0.13	100	0.97	0.03	0	100	0.99	0.01	99	0.82	0.02	0.05	0.12	0
Area 7																																		
FF. Muzzel		31	0.92	0.08	0	0	31	0.77	0.19	0.03	31	0.77	0.23	0	31	0.97	0.03	0	0	31	0.97	0.13	30	1.00	0	0	31	1.00	0	31	0.63	0.02	0.05	0.31	0
GG. Nawkon		53	0.92	0.08	0.005	0	53	0.81	0.14	0.05	53	0.84	0.14	0.02	53	0.99	0.10	0	0	53	0.92	0.08	53	0.98	0.02	0	53	0.99	0.01	53	0.81	0.01	0.03	0.15	0
Area 8 - Bella Coola River																																		
HH. Kilemik		99	0.88	0.12	0	0	100	0.89	0.27	0.04	99	0.87	0.13	0	100	1.00	0	0	0	99	0.93	0.17	99	0.99	0.01	0	100	0.99	0.01	100	0.79	0.04	0.03	0.15	0
Area 10																																		
II. Nakkin Channel		99	0.90	0.09	0.01	0	100	0.89	0.28	0.03	95	0.84	0.26	0	100	0.97	0.03	0	0	100	0.

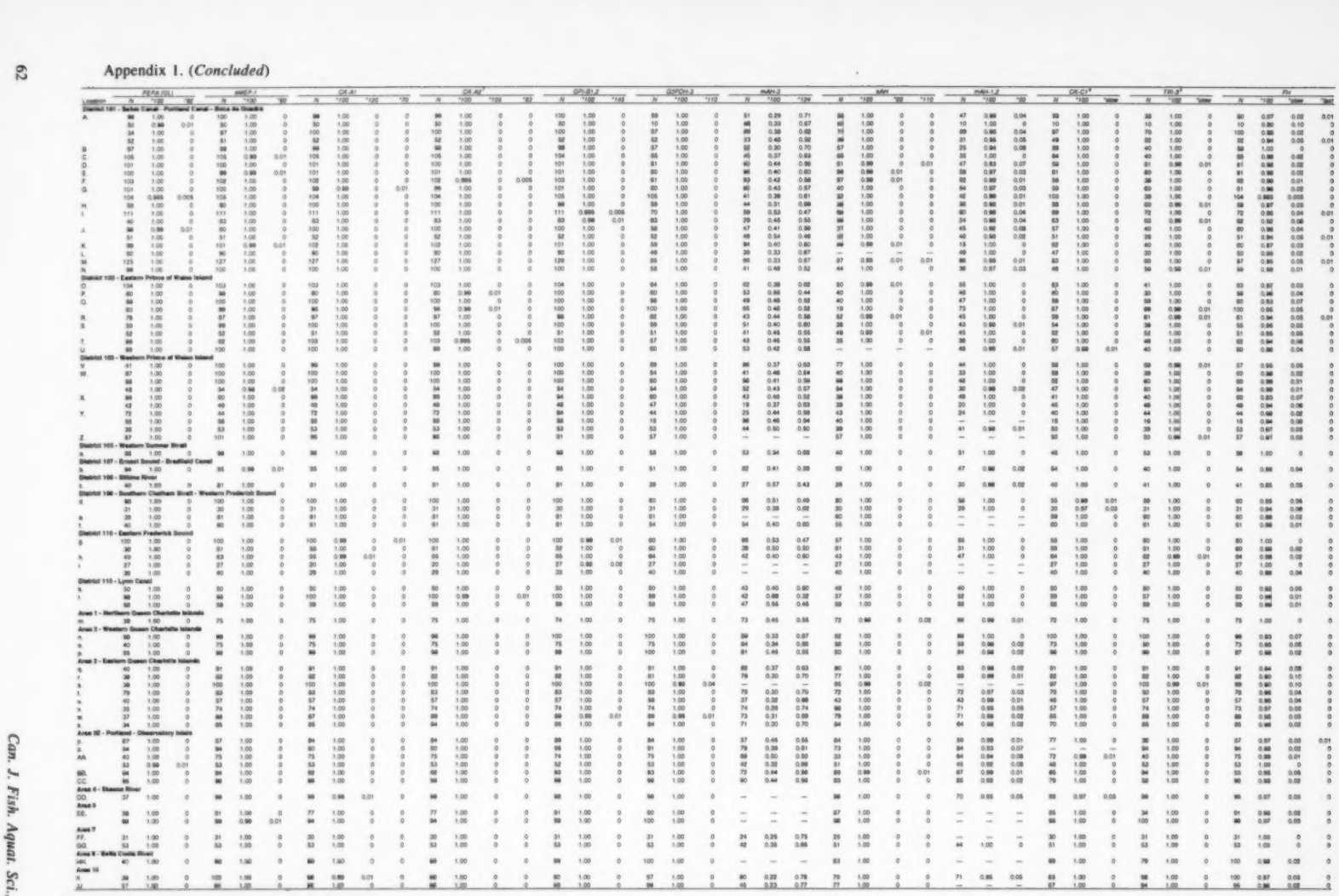
Appendix 1. (*Continued*)

Location	N	*100	*80	*87	*75	*135	*129	N	*100	*122	N	*100	*95	*105	N	*100	*90	*110	N	*100	*110	*70	N	*100	*90	N	*a	*b	*c	*d	*e	N	*100	*mid	R	*100	*ter
District 101 - Behm Canal - Portland Canal - Boca de Quadra																																					
A.	80	0.96	0.01	0	0.01	0	0	94	0.99	0.11	100	1.00	0	0	100	1.00	0	0	100	0.99	0.01	0	99	0.78	0.22	99	0.92	0.06	0	0.02	0	99	0.83	0.17	98	1.00	0
	80	0.96	0	0	0.04	0	0	49	0.78	0.22	50	1.00	0	0	99	1.00	0	0	50	1.00	0	0	49	0.83	0.17	10	0.90	0.10	0	0	0	99	0.84	0.12	10	1.00	0
	100	0.96	0	0	0.04	0	0	100	0.89	0.11	100	1.00	0	0	100	1.00	0	0	100	1.00	0	0	98	0.83	0.17	99	0.92	0.07	0	0.01	0	99	0.86	0.14	81	0.98	0.01
	92	1.00	0	0	0	0	0	51	0.88	0.12	52	1.00	0	0	52	1.00	0	0	52	1.00	0	0	92	0.80	0.20	92	0.92	0.08	0	0	0	51	0.78	0.22	52	1.00	0
	92	1.00	0	0	0	0	0	98	0.91	0.09	98	1.00	0	0	98	0.97	0.03	0.01	98	1.00	0	0	97	0.79	0.21	93	0.93	0.05	0	0.02	0	97	0.90	0.20	80	1.00	0
B.	58	0.98	0	0	0.01	0	0	98	0.91	0.09	98	1.00	0	0	98	0.99	0.01	0	98	1.00	0	0	98	0.82	0.18	93	0.95	0.05	0	0.01	0	98	0.83	0.17	40	0.99	0.01
C.	65	0.96	0	0	0.02	0.01	0.01	105	0.97	0.13	105	0.995	0.005	0	105	0.99	0.01	0	105	1.00	0	0	105	0.82	0.18	65	0.95	0.05	0	0.01	0	105	0.83	0.17	61	1.00	0
D.	100	0.97	0.01	0	0.02	0	0	100	0.91	0.09	100	1.00	0	0	101	1.00	0	0	101	1.00	0	0	100	0.83	0.17	61	0.93	0.06	0.01	0	0	99	0.85	0.20	61	1.00	0
E.	101	0.97	0	0	0.02	0.01	0.01	101	0.90	0.10	101	1.00	0	0	101	0.99	0.02	0	101	1.00	0	0	99	0.82	0.18	60	0.91	0.08	0.01	0	0	100	0.87	0.13	60	1.00	0
F.	102	0.96	0	0	0.02	0	0	101	0.90	0.10	103	0.996	0.005	0	103	0.99	0.01	0	102	1.00	0	0	99	0.80	0.20	63	0.91	0.09	0	0	0	103	0.92	0.08	84	1.00	0
G.	61	0.98	0	0	0.01	0	0	100	0.89	0.11	101	1.00	0	0	101	1.00	0	0	101	1.00	0	0	100	0.83	0.17	61	0.86	0.14	0	0	0	101	0.86	0.14	61	1.00	0
	105	0.98	0	0	0.02	0	0	104	0.92	0.08	105	0.995	0.005	0	105	1.00	0	0	105	1.00	0	0	105	0.75	0.25	105	0.85	0.15	0.005	0	0	104	0.86	0.14	40	1.00	0
H.	60	0.98	0	0	0.02	0	0	98	0.92	0.08	100	0.99	0.01	0.01	100	0.99	0.01	0	99	1.00	0	0	99	0.81	0.19	60	0.92	0.08	0	0	0	99	0.78	0.22	60	1.00	0
I.	111	0.98	0	0.005	0.02	0	0	111	0.92	0.08	111	0.995	0.005	0	111	0.97	0.03	0	111	1.00	0	0	109	0.78	0.22	72	0.89	0.11	0	0	0	111	0.88	0.12	72	1.00	0
	63	0.98	0	0	0.02	0	0	63	0.90	0.10	63	1.00	0	0	63	0.98	0.02	0	63	1.00	0	0	63	0.86	0.14	63	0.92	0.08	0	0	0	63	0.79	0.21	63	1.00	0
J.	59	0.97	0.02	0	0.02	0	0	100	0.93	0.07	100	1.00	0	0	100	0.97	0.03	0	99	1.00	0	0	97	0.80	0.11	59	0.92	0.08	0	0	0	99	0.86	0.14	40	1.00	0
	52	0.97	0	0	0.03	0	0	52	0.92	0.08	52	1.00	0	0	52	0.95	0.05	0	52	1.00	0	0	52	0.83	0.17	52	0.94	0.06	0	0	0	52	0.83	0.17	39	1.00	0
K.	102	0.97	0.01	0	0.02	0	0	102	0.99	0.11	102	1.00	0	0	102	0.99	0.01	0	101	1.00	0	0	99	0.84	0.16	62	0.93	0.10	0	0	0	101	0.82	0.18	40	1.00	0
L.	50	1.00	0	0	0	0	0	50	0.92	0.08	50	0.99	0.01	0.01	50	0.99	0.01	0	50	1.00	0	0	98	0.86	0.14	50	0.96	0.14	0	0	0	50	0.89	0.11	50	1.00	0
M.	87	0.94	0.02	0	0.02	0	0	127	0.99	0.11	127	1.00	0	0	127	0.992	0.004	0	127	1.00	0	0	104	0.84	0.16	87	0.95	0.05	0	0	0	104	0.83	0.17	60	1.00	0
N.	59	0.96	0.01	0	0.03	0	0	59	0.95	0.15	100	1.00	0	0	100	0.99	0.01	0	99	1.00	0	0	99	0.84	0.16	57	0.94	0.06	0	0	0	99	0.85	0.15	59	1.00	0
District 102 - Eastern Prince of Wales Island																																					
O.	94	0.94	0	0.01	0.05	0	0.01	102	0.97	0.03	104	0.97	0.01	0.01	104	0.995	0.005	0	103	1.00	0	0	104	0.80	0.20	64	0.91	0.09	0	0	0	104	0.92	0.08	41	1.00	0
P.	90	0.95	0	0	0.04	0	0.01	100	0.94	0.06	100	1.00	0	0	100	0.99	0.01	0	100	1.00	0	0	99	0.83	0.17	60	0.89	0.11	0	0	0	100	0.90	0.10	30	1.00	0
Q.	90	0.95	0	0.01	0.03	0	0.02	100	0.95	0.05	98	1.00	0	0	100	0.99	0.01	0	100	1.00	0	0	97	0.77	0.23	60	0.89	0.11	0	0	0	97	0.93	0.07	60	1.00	0
	100	0.93	0	0	0.05	0	0.01	99	0.93	0.07	98	0.99	0.01	0	100	0.99	0.01	0	100	1.00	0	0	100	0.70	0.30	100	0.91	0.09	0	0	0	99	0.93	0.07	100	1.00	0
R.	83	0.98	0	0	0.01	0	0	98	0.94	0.06	100	0.97	0.02	0.01	100	1.00	0	0	100	1.00	0	0	101	0.77	0.23	62	0.91	0.08	0	0	0.01	101	0.89	0.10	61	1.00	0
S.	89	0.96	0	0.01	0.03	0	0	100	0.95	0.05	100	0.97	0.03	0	100	0.99	0.01	0	100	1.00	0	0	97	0.81	0.19	62	0.89	0.11	0	0	0	99	0.92	0.08	58	1.00	0
	52	0.96	0	0	0.03	0	0.01	52	0.94	0.06	52	0.96	0	0.04	52	0.97	0.03	0	52	1.00	0	0	52	0.81	0.19	52	0.89	0.11	0	0	0	52	0.94	0.06	52	1.00	0
T.	92	0.96	0	0	0.03	0	0	103	0.95	0.05	103	0.98	0.01	0.01	103	1.00	0	0	103	1.00	0	0	96	0.76	0.24	58	0.89	0.11	0	0	0	58	0.95	0.05	48	1.00	0
U.	60	0.97	0	0	0.03	0	0	100	0.96	0.04	100	0.97	0.03	0	100	0.99	0.01	0	100	1.00	0	0	100	0.77	0.23	60	0.95	0.05	0	0	0	100	0.94	0.06	40	1.00	0
District 103 - Western Prince of Wales Island																																					
V.	100	0.97	0	0	0.03	0	0.01	96	0.95	0.05	100	0.98	0.01	0.01	100	0.99	0.02	0	99	1.00	0	0	96	0.85	0.15	59	0.87	0.12	0.01	0	0	99	0.95	0.05	59	1.00	0
W.	60	0.94	0	0	0.06	0	0	96	0.91	0.09	100	0.99	0.01	0	100	0.99	0.01	0	100	1.00	0	0	92	0.79	0.21	60	0.95	0.05	0	0	0	99	0.86	0.14	30	1.00	0
	100	0.91	0	0	0.09	0	0	99	0.91	0.09	100	0.99	0.01	0.01	100	0.99	0.01	0	99	1.00	0	0	100	0.74	0.26	60	0.93	0.07	0	0	0	100	0.94	0.06	60	1.00	0
	54	0.93	0	0	0.10	0	0	94	0.93	0.07	93	0.99	0.01	0	94	1.00	0	0	94	1.00	0	0	83	0.75	0.25	54	0.95	0.05	0	0	0	54	0.93	0.07	60	1.00	0
	60	0.93	0	0	0.10	0	0	103	0.93	0.07	99	1.00	0	0	100	1.00	0	0	100	1.00	0	0	90	0.81	0.19	60	0.86	0.14	0	0	0	98	1.00	0	40	1.00	0
X.	60	0.93	0	0	0.07	0	0	46	0.97	0.03	46	0.97	0.02	0.01	46	1.00	0	0	46	1.00	0	0	47	0.78	0.22	47	0.93	0.06	0	0	0.01	47	1.00	0	40	1.00	0
	49	0.96	0	0.01	0.04	0	0	49	0.94	0.06	45	0.96	0.04	0	49	0.98	0.02	0	49	1.00	0	0	47	0.81	0.19	47	0.94	0.06	0	0	0	47	0.96	0.04	40	1.00	0
Y.	44	0.93	0	0.01	0.09	0	0	93	0.95	0.05	93	0.96	0.01	0	94	0.96	0.02	0	94	1.00	0	0	83	0.80	0.20	44	0.95	0.05	0	0	0	44	0.93	0.07	44	1.00	0
	56	0.94	0	0.01	0.05	0	0	94	0.95	0.05	94	0.99	0	0.01	56	0.98	0.01	0	56	1.00	0	0	54	0.76	0.24	56	0.95	0.05	0	0	0	56	0.91	0.09	16	1.00	0
	53	0.91	0	0	0.09	0	0	52	0.97	0.13	47	1.00	0	0	53	0.99	0.01	0	53	1.00	0	0	53	0.68	0.32	53	0.89	0.11	0	0	0.01	53	0.96	0.02	39	1.00	0
Z.	53	0.94	0	0	0.06	0	0	99	0.96	0.02	97	0.96	0.01	0	101	0.99	0.01	0	101	1.00	0	0	97	0.80	0.20	60	0.87	0.13	0	0	0	99	0.87	0.13	40	1.00	0
District 105 - Western Summer Strait																																					
a.	99	0.97	0	0	0.03	0	0	96	0.91	0.09	96	0.96	0.01	0	99	0.96	0.05	0	98	1.00	0	0	99	0.74	0.26	86	0.78	0.22	0	0	0	97	0.91	0.09	51	1.00	0
District 107 - Ernest Sound - Bradfield Canal																																					
b.	80	0.99	0	0	0.01	0	0	93	0.89	0.12	94	1.00	0	0	99	0.99	0.01	0	95	1.00	0	0	91	0.86	0.14	83	0.83	0.17	0	0	0	93	0.81	0.19	40	1.00	0
District 106 - Stikine River																																					
c.	91	0.96	0	0	0.02	0	0	79	0.96	0.04	80	1.00	0	0	91	0.96	0.04	0	91	1.00	0	0	79	0.84	0.16	39	0.85	0.15	0	0	0	79	0.84	0.16	41	1.00	0
District 108 - Southern Chatham Strait - Western Frederick Sound																																					
d.	100	0.97	0	0	0.03	0	0	99	0.97	0.03	100	1.00	0	0	100	0.97	0.01	0.02	100	1.00	0	0	100	0.80	0.20	60	0.93	0.07	0	0	0	100	0.87	0.13	60	1.00	0
	31	0.95	0	0	0.05	0	0	30	0.95	0.05	31	1.00	0	0	31	0.94	0.03	0.03	31	1.00	0	0	31	0.81	0.19	31	0.90	0.10	0	0	0	31	0.87	0.13	31	1.00	0
e.	61	0.95	0	0	0.05	0	0	55	0.94	0.06	60	1.00	0	0	61	0.97	0.03	0	61	1.00	0	0	61	0.79	0.21	61	0.81	0.15	0.01	0	0	60	0.95	0.05	59	1.00	0
f.	61	0.96	0	0	0.04	0	0	60	0.97	0.03	59	0.98	0.01	0	61	0.95	0.03	0	61	1.00	0	0	55	0.78	0.22	61	0.84	0.16	0.01	0	0	61	0.84	0.16	61	1.00	0
District 110 - Eastern Frederick Sound																																					
g.	100	0.99	0	0	0.01	0	0	99	0.90	0.10	100	0.99	0	0.01	100	0.94	0.05	0.01	100	1.00	0	0	99	0.84	0.16	60	0.86	0.14	0	0	0	100	0.87	0.13	59	1.00	0
	61	0.93	0	0	0.07	0	0	61	0.95	0.05	40	1.00	0	0	61	0.97	0.03	0	61	1.00	0	0	59	0.79	0.21	61	0.87	0.13	0	0	0	59	0.86	0.14	59	1.00	0
h.	65	0.94	0.01	0	0.02	0	0	64	0.94	0.05	65	1.00	0	0	65	0.95	0.03	0	65	1.00	0	0	63	0.86	0.14	64	0.87	0.13	0	0	0	65	0.86	0.14	63	1.00	0
i.	37	0.96	0	0	0.04	0	0	34	0.94	0.06	27	1.00	0	0	27	1.00	0	0	27	1.00	0	0	27	0.67	0.33	27	0.85	0.11	0	0	0	27	0.93	0.07	27	1.00	0
	40	1.00	0	0	0	0	0	39	0.94	0.06	40	1.00	0	0	40	1.00	0	0	40	1.00	0	0	40	0.60	0.20	40	0.85	0.15	0	0	0	39	0.96	0.05	40	1.00	0
District 116 - Lynn Canal																																					
k.	80	0.96	0	0	0.02	0	0	80	0.91	0.09	80	1.00	0	0	80	0.95	0.05	0	80	1.00	0	0	80	0.88	0.12	80	0.87	0.13	0	0	0	80	0.82	0.18	80	1.00	0
l.	100	0.97	0	0	0.03	0	0	100	0.92	0.08	100	0.99	0.01	0	100	0.99	0.01	0	99	1.00	0	0	99	0.84	0.16	99	0.89	0.11	0	0	0	98	0.79	0.22	57	1.00	0
	59	0.95	0	0	0.05	0	0	59	0.97	0.03	59	0.98	0.02	0	59	1.00	0	0	59	1.00	0	0	59	0.81	0.19	59	0.84	0.16	0	0	0	59	0.71	0.29	59	1.00	0
Area 1 - Northern Queen Charlotte Islands																																					
m.	75	0.99	0	0	0.01	0	0.01	75	0.99	0.01	75	1.00	0	0	75	0.86	0.14	0	75	0.99	0	0.01	75	0.94	0.06	75	0.97	0.03	0	0	0	75	0.85	0.15	75	1.00	0
Area 2 - Western Queen Charlotte Islands																																					
n.	100	0.96	0	0.01	0.12	0	0.01	99	0.92	0.18	99	1.00	0	0	99	0.98	0.02	0	99	1.00	0	0	99	0.77	0.23	100	0.94	0.04	0.01	0	0.01	99	0.86	0.14	100	1.00	0
o.	75	0.97	0	0	0.03	0	0	75	0.99	0.11	75	1.00	0	0	75	0.99	0.01	0	75	1.00	0	0	74	0.87	0.13	75	0.95	0.05	0.01	0	0	75	0.91	0.09	50	1.00	0
p.	100	0.95	0	0	0.05	0	0.01	99	0.91	0.19	99	1.00	0	0	100	0.91	0.09	0	99	1.00	0	0	100	0.89	0.11	99	0.90	0.04	0.07	0	0	100	0.95	0.05	99	0.99	0.01
Area 3 - Eastern Queen Charlotte Islands																																					
q.	91	0.92	0	0	0.08	0	0	91	0.93	0.17	87	1.00	0	0	91	0.97	0.03	0	90	1.00	0	0	91	0.88	0.12	91	0.87	0.12	0.01	0	0	91	0.97	0.03	91	1.00	0
r.	82	0.95	0	0	0.04	0	0.01	82	0.90	0.11	82	1.00	0	0	82	0.96	0.04	0	82	1.00	0	0	81	0.89	0.11	81	0.91	0.09	0	0	0	82	0.91	0.09	82	1.00	0
s.	100	0.91	0	0	0.09	0	0.01	100	0.90	0.10	98	1.00	0	0	100	0.95	0.05	0	100	0.99	0	0.01	98	0.90	0.10	100	0.93	0.05	0	0	0.01	100	0.91	0.09	100	1.00	0
t.	93	0.93	0	0	0.09	0	0.01	93	0.91	0.09	92	1.00	0	0	93	0.93	0.01	0	93	1.00	0	0	92	0.87	0.13	93	0.92	0.04	0	0	0.04	93	0.93	0.07	93	1.00	0
u.	57	0.95	0	0	0.04	0	0.01	57	0.93	0.07	55	1.00	0	0	57	0.93	0.01	0	57	1.00	0	0	54	0.83	0.17	55	0.89	0.13	0	0	0.02	55	0.89	0.11	57	1.00	0
v.	74	0.93	0	0.01	0.05	0	0.01	74	0.91	0.09	74	1.00	0	0	74	0.95	0.05	0	74	1.00	0	0	73	0.84	0.16	74	0.95	0.05	0.01	0	0.01	73	0.86	0.13	74	1.00	0
w.	89	0.93	0	0	0.05	0	0.02	85	0.94	0.16	89	1.00	0	0	89	0.94	0.06	0	89	1.00	0	0	88	0.88	0.14	89	0.92	0.05	0	0	0.03	89	0.93	0.07	89	1.00	0
x.	85	0.96	0	0.01	0.02	0	0.01	85	0.89	0.11	85	1.00	0	0	85	0.95	0.04	0	85	1.00	0	0	84	0.83	0.17	84	0.92	0.05	0	0	0.02	84	0.89	0.11	85	1.00	0
Area 32 - Portland - Observatory Inlets																																					
y.	88	0.97	0	0	0.03	0	0	85	0.89	0.11	85	1.00	0	0	88	0.99	0.01	0	88	1.00	0	0	85	0.82	0.18	88	0.91	0.09	0	0	0	87	0.77	0.23	40	1.00	0
z.	95	0.96	0.01	0.01	0.04	0	0	95	0.85	0.15	92	1.00	0	0	95	0.99	0.01	0	95	1.00	0	0	90	0.79	0.21	94	0.87	0.10	0	0.04	0	93	0.82	0.18	94	0.98	0.02
AA.	75	0.94	0.01	0	0.05	0	0	75	0.93	0.17	75	1.00	0	0	75	0.99	0.01	0	75	0.99	0.01	0	74	0.86	0.14	75	0.91	0.09	0	0	0	73	0.86	0.14	33	1.00	0
	53	0.91	0.02	0	0.06	0	0	53	0.92	0.08	52	1.00	0	0	53	0.99	0.01	0	53	0.98	0.02	0	53	0.87	0.13	53	0.89	0.11	0	0.01	0	53	0.81	0.19	53	0.98	0.01
BB.	94	0.96	0.01	0	0.02	0.01	0	93	0.85	0.15	94	0.98	0.02	0	94	0.99	0.02	0	94	1.00	0	0	91	0.78	0.21	94	0.88	0.12	0	0	0	94	0.77	0.23	94	1.00	0
CC.	98	0.93	0	0	0.06	0	0	98	0.93	0.07	98	1.00	0	0	98	0.99	0.01	0	98	1.00	0	0	98	0.90	0.10	98	0.92	0.06	0	0	0	98	0.84	0.16	58	1.00	0
Area 4 - Skeena River																																					
DD.	99	0.94	0.04	0	0.02	0	0	97	0.94	0.06	94	1.00	0	0	99	0.96	0.02	0	99	1.00	0	0	96	0.94	0.06	99	0.94	0.06	0	0	0	99	0.93	0.07	99	1.00	0
Area 6																																					
EE.	95	0.98	0	0	0.02	0	0	93	0.87	0.13	19	1.00	0	0	95	1.00	0	0	92	1.00	0	0	87	0.87	0.13	92	0.93	0.07	0	0	0	92	0.85	0.15	34	1.00	0
	100	0.97	0.01	0	0.03	0	0	100	0.83	0.17	97	1.00	0	0	100	0.99	0.01	0	100	1.00	0	0	99	0.86	0.14	100	0.91	0.09	0	0.01	0	100	0.71	0.29	99	1.00	0
Area 7																																					
FF.	31	1.00	0	0	0	0	0	31	0.87	0.13	31	1.00	0	0	31	0.95	0.05	0	31	1.00	0	0	31	0.81	0.19	31	0.89	0.11	0	0	0	31	0.84	0.16	31	0.98	0.02
GG.	53	0.96	0	0.02	0.02	0	0	53	0.76	0.24	52	1.00	0	0	53	1.00	0	0	53	1.00	0	0	53	0.82	0.18	53	0.85	0.15	0	0	0	53	0.85	0.15	53	1.00	0
Area 9 - Stella Creek River																																					
HH.	100	0.97	0.01	0	0.01	0	0	99	0.84	0.16	97	0.99	0.01	0	100	1.00	0	0	99	1.00	0	0	100	0.89	0.11	100	0.95	0.05	0	0	0	99	0

Appendix 1. (*Continued*)



Appendix 1. (*Concluded*)



[1]One fast heterozygote (**100/*140*) found at Mussel was pooled with the common allele.

[2]We found one and three slow heterozygotes at Nekite Channel and Nekite River, respectively; one fast heterozygote at Traitors 1988; one fast heterozygote at Elena Bay; one slow heterozygote at *LDH-A2** at Kitimat 1988. These variants were pooled with the common allele.

[3]Two buffers are required to resolve alleles; mobilities for alleles "*a*, *b*, *c*, *d*, and *e*" are **−100* and **100*, **−126* and **100*, **−127* and **59*, **−146* and **59*, and **−72* and **110*, for gel buffers CA5.75 and TG (or R), respectively.

[4]Two buffers are required to resolve alleles; mobilities for alleles "*a*, *b*, *c*, *d*, and *e*" are **100* and **100*, **92* and **94*, **92* and **91*, **81* and **86*, and **86* and **94* for gel buffers CA7 and MF, respectively.

[5]Phenotypic frequencies listed: **100* includes both **−100/*−100* and **−100/*null* phenotypes; **null* includes only **null/*null* phenotypes. One **−100/*−180* heterozygote at Disappearance 1988 and one **−180/*−180* or **−180/*null* phenotype at Disappearance 1988 were found. These variants were pooled with the common allele.

[6]Four heterozygotes (**100/*125*) found at Herman Creek 1990 were pooled with the common allele.

[7]One very slow heterozygote found at Tombstone 1986 was pooled with the common allele.

[8]Three slow *CK-C2** heterozygotes found at Donkey 1990 were pooled with the common allele.

[9]Two fast heterozygotes found at Breezy Bay 1987 were pooled with the common allele.

[a]Two fast heterozygotes found at Breezy Bay 1987 were pooled with the common allele.

APPENDIX 2. Log-likelihood ratio analysis: regional comparisons of chum salmon from southeast Alaska and northern British Columbia.

		Within Regions								
Locus		South SE AK. Northern B.C.	Prince of Wales Is.	Central/ Northern SE	NW Queen Charlotte	SE Queen Charlotte Is.	Central BC	Total within	Total among	Total
*sAAT-1,2**	df	38	22	18	10	10	12	110	10	120
	G	24.96	9.32	86.93***	39.69***	4.67	14.73	180.30***	211.45***	391.75***
*mAAT-1**	df	38	22	18	10	10	12	110	10	120
	G	73.11***	78.28***	47.91***	62.59***	13.17	91.75***	366.81***	567.21***	934.02***
*ALAT**	df	38	22	18	10	10	12	110	10	120
	G	69.79**	36.50*	26.95	57.84***	9.71	49.26***	250.05***	98.19***	348.24***
*ESTD-1**	df	38	22	18	10	10	12	110	10	120
	G	19.94	52.98***	18.85	23.98**	11.90	31.49**	159.14**	294.19***	453.33***
*mIDHP-1**	df	19	11	9	5	5	6	55	5	60
	G	32.46	31.38***	9.62	15.10**	5.81	36.93***	131.30***	236.35***	367.65***
*LDH-A1**	df	38	22	18	10	10	12	110	10	120
	G	17.05	31.27	6.04	12.55*	10.48	29.67**	107.06	70.65***	177.71***
*LDH-B2**	df	19	11	9	5	5	6	55	5	60
	G	34.40	14.18	17.76*	46.38***	2.54	6.90	122.16***	215.65***	337.81***
*PEPB-1**	df	76	44	36	20	20	24	220	20	240
	G	72.26	42.75	45.36	44.47**	18.33	67.46***	290.63***	283.71***	574.34***
*sMDH-B1,2**	df	76	44	36	20	20	24	220	20	240
	G	98.37***	62.26*	11.83	42.21**	22.28	51.04**	287.99**	166.79***	454.78***
*mMEP-2**	df	19	11	9	5	5	6	55	5	60
	G	31.92*	20.92*	16.68	45.70***	6.87	26.70***	148.79***	155.25***	304.04***
*GPI-A**	df	38	22	18	10	10	12	110	10	120
	G	32.45	42.65**	18.34	0	0	3.63	97.07	85.84***	182.91***
*PGDH**	df	38	22	18	10	10	12	110	10	120
	G	60.17*	16.87	50.55***	41.88***	10.45	17.78	197.70***	131.84***	329.54***
*G3PDH-2**	df	19	11	9	5	5	6	55	5	60
	G	30.77*	20.45*	13.36	24.58***	5.18	17.28**	111.62***	95.97***	207.59***
*MPI**	df	76	44	36	20	20	24	220	20	240
	G	80.42	31.55	22.14	55.04***	25.48	19.38	234.01	254.73***	488.74***
*PGM-1**	df	19	11	9	5	5	6	55	5	60
	G	20.07	31.64***	25.88**	12.28*	2.33	34.74***	126.94***	140.11***	267.05***
*PGM-2**	df	38	22	18	10	10	12	110	10	120
	G	25.29	12.98	26.12	0	0	26.27**	90.66	39.47***	130.13
*sMDH-A1**	df	19	11	9	5	5	6	55	5	60
	G	30.97*	20.89*	10.31	22.37***	7.37	25.17***	117.08***	174.43***	291.51***
*sMDH-A2**	df	19	11	9	5	5	6	55	5	60
	G	51.18***	29.85**	12.57	8.39	2.83	12.08	116.90***	101.38***	218.28***
*sAAT-3**	df	38	22	18	10	10	12	110	10	120
	G	59.01*	18.69	67.20***	14.40	19.63*	28.37**	207.30***	314.96***	522.26***
*LDH-C**	df	19	11	9	5	5	6	55	5	60
	G	20.00	0	6.99	0	0	22.19**	49.18	49.56***	98.74**
*CK-B**	df	19	11	9	5	5	6	55	5	60
	G	11.87	6.76	0	13.37*	3.50	0	35.50	10.43	45.93
*TPI-1**	df	19	11	9	5	5	6	55	5	60
	G	0	0	15.41	0	0	0	15.41	19.77**	35.18

APPENDIX 2. (*Concluded*)

Locus		Within Regions: South SE AK. Northern B.C.	Prince of Wales Is.	Central/ Northern SE	NW Queen Charlotte	SE Queen Charlotte Is.	Central BC	Total within	Total among	Total
*IDDH-2**	df	19	11	9	5	5	6	55	5	60
	G	5.55	6.74	5.65	6.32	3.39	7.12	34.77	2.81	37.58
*sMEP-1**	df	19	11	9	5	5	6	55	5	60
	G	18.31	7.11	0	0	0	2.58	28.00	6.07	34.07
*GPI-B1,2**	df	19	11	9	5	5	6	55	5	60
	G	10.50	0	6.29	0	3.40	0	20.19	6.61	26.80
*G3PDH-3**	df	19	11	9	5	5	6	55	5	60
	G	0	0	0	0	22.66***	0	22.66	42.90***	65.56
*TPI-4**	df	19	11	9	5	5	6	55	5	60
	G	18.14	0	0	3.26	0	9.23	30.63	12.85*	43.48
*FH**	df	38	22	18	10	10	12	110	10	120
	G	51.33	25.57	23.38	31.13***	12.98	7.70	152.09**	53.13***	205.22***
Total	df	893	517	423	235	235	282	2585	235	2820
	G	1000.29**	651.59***	592.12***	623.53***	224.96	639.45***	3731.94***	3842.30***	7714.35***

* = P <0.05, ** = P <0.01, *** = P <0.001.

Genetic Diversity Patterns of Chum Salmon in the Pacific Northwest

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We used starch-gel electrophoresis to examine over 13 000 adult chum salmon, *Oncorhynchus keta*, from 153 collections at 105 locations in Washington, Oregon, and southern British Columbia from 1985–92. We identified 39 variable loci and 36 monomorphic loci. In general, alleles that occurred at a frequency >10% were found in all locations and were temporally stable within locations. Localized alleles usually occurred at a frequency <6%. Significant allele frequency differences were found among chum salmon populations with different spawning times (summer, fall, and winter), even within the same watershed, and among populations of the same spawning time in different regions. Significant among-population diversity was also found within regions. Multidimensional scaling and UPGMA cluster analysis grouped populations by geography and run-timing, except where past stock transfers have resulted in interbreeding of hatchery and wild fish. These analyses indicate that both geography and spawning time are important isolating mechanisms and that, compared with the other populations, summer-run chum salmon from the Strait of Juan de Fuca and Hood Canal are a distinct evolutionary lineage. This information is currently being used to conserve the genetic diversity of wild chum salmon and manage mixed-stock fisheries.

Nous avons utilisé l'électrophorèse sur gel d'amidon pour examiner plus de 13 000 saumons kéta adultes (*Oncorhynchus keta*) provenant de 153 collections conservées en 105 lieux dans les états de Washington et de l'Oregon et dans le sud de la Columbie-Britannique entre 1985 et 1992. Nous avons identifié 39 loci variables et 36 loci monomorphes. En général, les allèles dont la fréquence était supérieure à 10% étaient présents dans tous les lieux et ils étaient temporairement stables à l'intérieur du lieu. La fréquence des allèles locaux était habituellement inférieure à 6%. Des différences appréciables de la fréquence allélique ont été observées entre les populations de saumon kéta dont les périodes de montaison étaient différentes (été, automne et hiver), même à l'intérieur du même bassin hydrographique, et parmi les populations de différentes régions dont la période de montaison était la même. Une diversité intra-population appréciable a également été notée à l'intérieur des régions. L'analyse multidimensionnelle et l'analyse typologique (par la méthode des moyennes arithmétiques non pondérées portant sur des échantillons appariés) ont permis de regrouper les populations selon la distribution géographique et la période de montaison, sauf lorsque des transferts de stock antérieurs avaient entraîné des croisements entre les poissons d'écloserie et les poissons sauvages. Ces analyses indiquent que la distribution géographique et la période de montaison constituent toutes deux des mécanismes d'isolement importants et que le saumon kéta à montaison estivale du détroit Juan de Fuca et celui du canal Hood représentent des lignées évolutives distinctes des autres populations. Cette information est actuellement appliquée à la conservation de la diversité génétique du saumon kéta sauvage et à la gestion des stocks mixtes.

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Chum salmon, *Oncorhynchus keta*, populations in the Pacific Northwest (Washington and Oregon, USA, and southern British Columbia, Canada) inhabit the numerous environmentally diverse streams along the Pacific coast, Puget Sound, and Georgia Strait during the freshwater phase of their anadromous life. Chum salmon in this study area are a component of a biologically diverse species that ranges from California to Alaska and the Northwest Territories of Canada in the eastern Pacific Ocean and, in Asia, from the Arctic coast of Russia to Korea (Salo 1991). In our study area, adults typically return to their natal stream at age 3–5 yr and spawn throughout a 6-mo period (September–February). Stream habitat varies from major river systems such as the Fraser River in British Columbia, to small streams with headwaters close to salt water. The species is able to use this wide variety of habitat because the fry migrate directly to salt water after emerging from the gravel. A diversity of life-history traits and local adaptations have been documented (Beacham and Murray 1987; Salo 1991). The strong homing instincts of Pacific salmon coupled with the diverse geography of the Pacific Northwest have the potential to promote a large amount of population genetic structuring in chum salmon.

Several million chum salmon are harvested annually by commercial fisheries in Washington and British Columbia. Because flesh quality decreases markedly once the fish enter freshwater, the majority of chum salmon harvest occurs in marine areas where different spawning populations are mixed. Many of these marine fisheries intercept fish bound for numerous locations in both the United States and Canada.

Fishery managers endeavor to make the harvest equitable for both countries according to the provisions of the Pacific Salmon Treaty and allocate catch among treaty Indian tribes in Washington and nontreaty fishing gear types (Graves 1989). The impacts to individual populations depend not only on their productivity, but on the timing and location of fisheries. Thus, a classical fisheries management problem exists on how to maximize harvest of productive populations without damaging less productive populations and how to spread harvest among fishers fairly.

The stock concept of fisheries management was developed to conserve species and manage mixed-stock fisheries (Simon and Larkin 1972; Berst and Simon 1981). The basis of the stock concept is that a species is divided into numerous discrete reproductive entities (stocks) within which interbreeding occurs with few restrictions, but that are reproductively isolated (somewhat) from other members of the same species. The strong homing instinct of Pacific salmon and differences in spawning times leads to the formation of stocks. Genetic differences among stocks are not simply related to distance, but tend to have distinct breaks that form a hierarchical structure reflecting past colonization, geography, and the length of time populations have been isolated. Therefore, effective use of the stock concept in fisheries management and conservation requires an understanding of genetic diversity patterns within and among stocks.

A fisheries management tool referred to as genetic stock identification (GSI) and more recently as mixed stock analysis (MSA) (Utter and Ryman 1993) has been applied to identify groups of stocks in fisheries. The application of this tool requires genetic characterization of fish caught in a fishery and of fish from spawning populations that are likely to be represented in the fishery. An estimate of the contribution of spawning populations and stock groups can be made from several maximum-likelihood models (Fournier et al. 1984; Millar 1987; Wood et al. 1987; Pella and Milner 1987). The basis for this tool is the detection of significant differences in the genetic attributes of the populations of fish estimated by the mixture analysis. When these differences exist, MSA has several advantages over other stock identification tools because these genetic marks are heritable, stable attributes of a population (Milner et al. 1985; Altukhov and Salmenkova 1987; Waples et al. 1990; Utter and Ryman 1993). The stock composition of chum fisheries in British Columbia and Washington has been estimated by MSA for fisheries management since 1981 (Fournier et al. 1984; Beacham et al. 1985, 1987; Shaklee et al. 1990b, 1990c; Phelps et al. 1991). Because chum salmon migrate to salt water immediately after emergence and the majority of the fish are from wild production, other common stock identification techniques such as scale-pattern analysis and coded-wire tagging are not practical tools for determining the origin of fish in mixed-stock fisheries.

In addition to fisheries, wild chum salmon populations have been impacted by the loss and degradation of habitat, and by hatchery production (Ricker 1972; Palmisano et al. 1993). The long-term productivity and viability of many populations are considered at risk (Nehlsen et al. 1991; WDF et al. 1993). Knowledge of stock origins and relationships are essential for conservation and restoration.

To increase harvest and rebuild impacted populations, hatchery propagation of chum salmon in Washington has occurred since 1913 (Washington Department of Fish and Wildlife hatchery records). Unfortunately, a typological concept of species existed until recently and considerable movement of eggs and fry from one geographical region to another has occurred. Most of the stock transfers have occurred from chum salmon hatcheries in Hood Canal to streams and hatcheries in south and north Puget Sound, and the Strait of Juan de Fuca. Although these transfers ceased in the early 1980s, hatchery strains (with the Hood Canal chum salmon gene pools) are still being used at some hatcheries and wild populations may have been mixed with hatchery strains at the hatchery and through straying. Recently, the hatching of chum salmon in small stream-side incubators has become popular with volunteer groups. When eggs are provided from hatchery sources, these projects have the potential to disrupt historic patterns of genetic diversity. The electrophoretic characterization of chum collections can be used to identify their genetic origin and the impact of nonlocal introductions (Altukhov and Salmenkova 1987, 1990). In addition, repeat sampling at a hatchery can identify temporal changes in allele frequencies, indicative of rapid genetic drift or selection and changes in the broodstock origin (Waples and Teel 1990).

The purpose of this study was to determine the amount and distribution of allelic variation in chum salmon populations from the Pacific Northwest and to use this information to identify genetic relationships among populations. An understanding of the mechanisms of these relationships, such as the relative importance of geographic isolation and spawning time as barriers to gene flow, will help guide the conservation of wild chum salmon populations and the management of commercial fisheries.

Materials and Methods

We made 153 collections for a total of over 13 000 post-spawning chum salmon adults between 1985 and 1992 from 105 run-timing – spawning-area aggregations, which are referred to as populations throughout this paper (Table 1, Fig. 1). The individual populations were grouped in geographical regions based on fishery management areas and geography. The geographical regions (and population numbers) referred to in this paper are: southern Puget Sound (1–21), Hood Canal (22–47), northern Puget Sound (48–65), Strait of Juan de Fuca (66–72), Washington and Oregon Pacific coast (73-80), Georgia Strait (81–92), Fraser River (93–102) and the west coast of Vancouver Island (103–105).

We collected naturally spawning adults from their spawning areas opportunistically with hook and line or with nets. Hatchery samples were taken from the pool of carcasses after spawning. Wild chum salmon spawning areas were located with assistance from state, tribal, and federal stock assessment and habitat biologists. When it was practical to do so, we took equal numbers of males and females, and sampled each spawning population several times during the spawning season to provide representation of early-, peak-, and late-spawning components. In several instances we focused our sampling on the extremes of the spawning period to allow comparisons of allele frequencies from the early and late portions of the run. We also made collections from 30 locations in more than 1 yr to examine the stability of allele frequencies over time.

The fish were immediately killed, bled, and approximately 1 cm^3 each of skeletal muscle, heart, liver, and retina

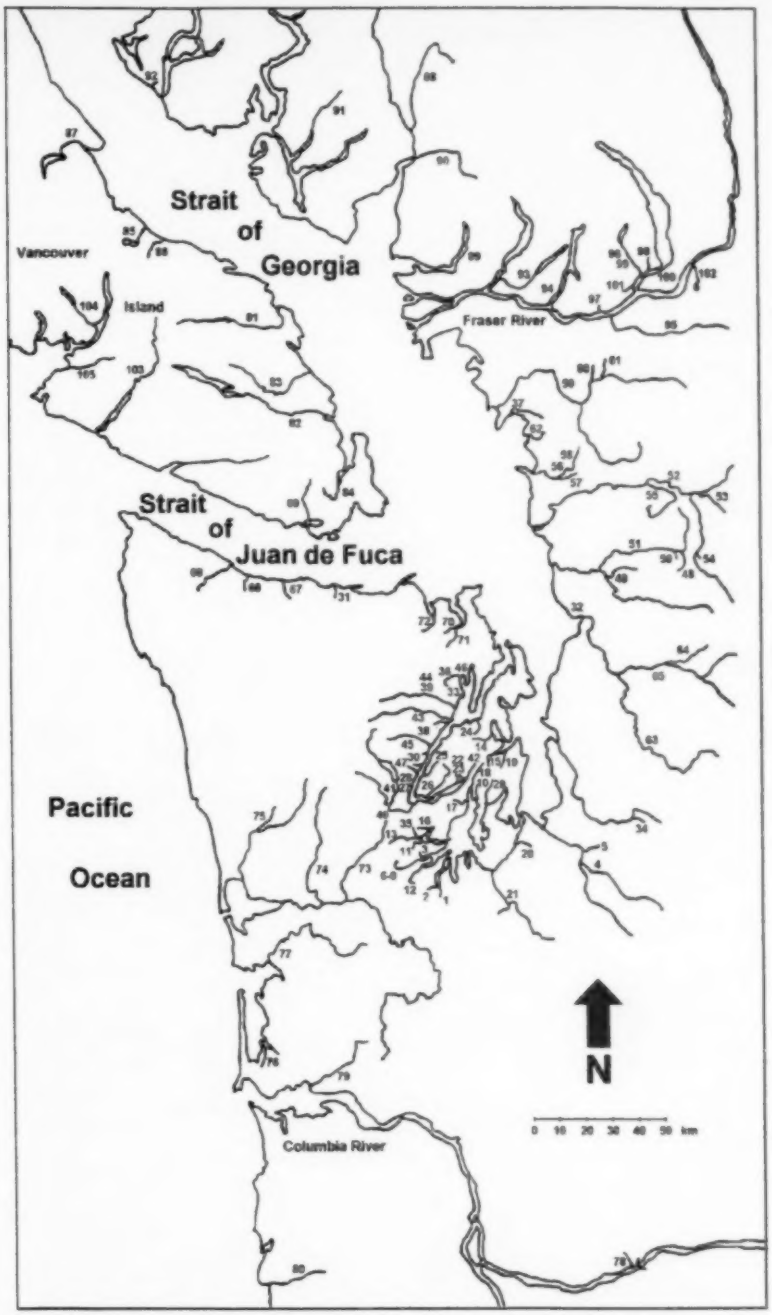

FIG. 1. Locations of chum salmon collections in southern British Columbia, Canada, and Washington and Oregon, USA. Numbers correspond to location names in Table 1.

suspended in vitreous humor was removed from each fish and put in individually labeled 12 × 75 mm plastic culture tubes. The heart, liver, and muscle samples were rinsed in clean water to remove superficial contaminants. We extracted retinal tissue from each fish by scraping the posterior of the eyeball with a 14-gauge hypodermic needle while drawing the vitreous humor (with suspended retinal tissue) into a syringe. All tissue samples were placed on dry ice immediately after dissection until they could be transferred to an ultra-freezer for storage at −70°C.

TABLE 1. Chum salmon sampling locations divided into genetic-origin regions, run-timing groups within a region, and subregions within a run-timng group. The significance of the chi-square test of allelic heterogeneity among populations within groups is given after each group subdivision. Sample size (N), within-population genetic variation measures (percentage of the 38 loci polymorphic at the 1 and 5% criterion levels), and average heterozygosity (direct count) based on the loci listed in the Appendix (except that *sAAT-3** was excluded because of missing data) for 105 chum salmon populations. FH = fish hatchery.

Location and run timing	N	% loci polymorphic ≥1%	% loci polymorphic ≥5%	Average heterozygosity
Southern Puget Sound				
Fall run: $P < 0.0001$				
Southern: $P < 0.0001$				
1. Swift Creek	250[a]	44.7	23.7	0.101
2. Perry Creek	350[a]	39.5	26.3	0.102
3. Elson Creek FH	350[a]	39.5	26.3	0.099
4. Carbon River	150	44.7	28.9	0.103
5. Fennel Creek	100[b]	39.5	26.3	0.093
6. Skookum Creek	100	34.2	26.3	0.108
7. Little Creek	51	36.8	26.3	0.099
8. Reitdorf Creek	50	34.2	26.3	0.098
9. Upper Skookum Creek	100	36.8	26.3	0.107
10. Sherwood, Rocky and Coulter creeks	106[b]	39.5	26.3	0.106
11. Mill Creek	179[a]	39.5	26.3	0.100
12. Kennedy Creek	200[a]	36.8	26.3	0.103
13. Goldsborough Creek	200	36.8	26.3	0.101
Kitsap Peninsula: $P = 0.066$				
14. Chico Creek	196[a]	36.8	26.3	0.104
15. Gorst Creek	100	31.6	28.9	0.113
Mean		38.1	26.5	0.102
Summer-run: $P < 0.0001$				
Southern: $P < 0.0001$				
16. Johns Creek FH	100	42.1	26.3	0.100
17. Sherwood Creek	100	34.2	28.9	0.107
18. Coulter Creek FH	100	36.8	26.3	0.103
Kitsap Peninsula				
19. Blackjack Creek	100	39.5	26.3	0.097
Mean		38.2	27.0	0.102
Winter-run: $P < 0.0001$				
20. Chambers Creek FH	100	36.8	26.3	0.092
21. Nisqually River	300[a]	42.1	28.9	0.097
Mean		39.5	27.6	0.095
Hood Canal				
Fall-run: $P < 0.0001$				
Eastern: $P < 0.0001$				
22. Big Mission Creek	67	34.2	28.9	0.110
23. Little Mission Creek	53	36.8	28.9	0.112
24. Big Beef Creek	100	42.1	26.3	0.104
25. Dewatto River	263[a]	52.6	26.3	0.103
26. Tahuya River	83[a]	47.4	26.3	0.108
Western: $P < 0.0001$				
27. Enetai FH	100	44.7	28.9	0.105
28. Hood Canal FH	450[a]	47.4	26.3	0.105
30. Lilliwaup Creek	100	39.5	26.3	0.101
33. Walcott FH	100	34.2	26.3	0.116
35. McKernan FH	100	44.7	26.3	0.103
36. Big Quilcene FH	100	39.5	28.9	0.116
38. Hamma Hamma River	186[a]	47.4	26.3	0.108
39. Dosewallips River	105	39.5	26.3	0.114
Skokomish wild: $P = 0.10$				
40. Vance Creek	101	34.2	26.3	0.110
41. NF Skokomish River	111	52.6	26.3	0.111
Hatcheries in other regions				
29. Minter Creek FH	100[b]	39.5	26.3	0.109
31. Lower Elwha FH	100[b]	39.5	26.3	0.107
32. Tulalip FH	197[a,b]	44.7	26.3	0.106
34. Keta Creek FH	150[a,b]	36.8	26.3	0.108
37. Bellingham Maritime FH	100[b]	47.4	26.3	0.100
Mean: wild populations		42.6	26.8	0.108
Mean: hatchery populations		41.8	26.8	0.108
Summer-run $P < 0.0001$				
Eastern				
42. Union River	103	34.2	28.9	0.099
Western: $P < 0.0001$				
43. Duckabush River	125[a]	36.8	31.6	0.101
44. Dosewallips River	102	34.2	28.9	0.096
45. Hamma Hamma River	37	31.6	31.6	0.100
46. Quilcene Bay & FH	240	39.5	31.6	0.096
47. Lilliwaup Creek	120[a]	34.2	28.9	0.102
Mean		42.5	27.0	0.108
Northern Puget Sound				
Fall-run: $P < 0.0001$				
Stillaguamish River: $P = 0.016$				
48. Squire Creek	140[a]	50.0	26.3	0.098
49. Jim Creek	151[a]	47.4	26.3	0.086
50. Fortson Creek	211[a]	50.0	26.3	0.099
51. N. F. Stillaguamish River	140[a]	42.1	26.3	0.093
Skagit River: $P < 0.001$				
52. Skagit River	100	47.4	26.3	0.099
53. Illabot Creek	98	39.5	26.3	0.094
54. Dan Creek	153[a]	50.0	26.3	0.101
55. Finney Creek	41	31.6	21.1	0.081
Samish River: $P = 0.06$				
56. Samish FH	100[a,b]	42.1	26.3	0.098
57. Thomas Creek	101[a]	47.4	26.3	0.095
58. Bob Smith Creek	100	44.7	26.3	0.094
Nooksack River: $P = 0.02$				
59. Nooksack River	36	26.3	26.3	0.077
60. Kendall FH	200[a]	47.4	26.3	0.089
61. Maple Creek	100	34.2	26.3	0.090
Bellingham Bay				
62. Chuckanut Creek	35	36.8	26.3	0.103
Skykomish River: $P = 0.01$				
63. Schoolhouse Slough	100	42.1	26.3	0.084
64. Wallace River	144[a]	55.3	26.3	0.098
65. Skykomish River	200[a]	52.6	26.3	0.102
Mean		43.8	26.0	0.093
Strait of Juan de Fuca				
Fall-run: $P < 0.0001$				
66. Deep Creek	100	36.8	23.7	0.101
67. Lyre River	100	36.8	21.1	0.083
68. Pysht River	255[a]	55.3	23.7	0.080
69. Sooke River	100	47.4	23.7	0.097
Mean		44.1	23.1	0.090
Summer-run: $P < 0.0001$				
70. Salmon Creek	50	34.2	23.7	0.100
71. Snow Creek	50	28.9	28.9	0.087
72. Jimmycomelately Creek	100	36.8	28.9	0.100
Mean		33.3	27.2	0.096
Coastal and Columbia River				
Fall-run: $P < 0.0001$				
Chehalis River: $P = 0.11$				
73. Satsop River	100	34.2	26.3	0.088
74. Wynoochee River	22	31.6	23.7	0.102
75. Stevens Creek	100	36.8	28.9	0.092
Willapa Bay: $P = 0.03$				
76. Ellsworth Creek	100	42.1	26.3	0.098
77. Bitter Creek	100	42.1	26.3	0.090
Columbia River: $P < 0.0001$				
78. Hamilton Creek	100	39.5	21.1	0.088
79. Grays River	100	42.1	28.9	0.094
Oregon Coast				
80. Coal Creek	100	39.5	28.9	0.104
Mean		38.5	26.3	0.095
Georgia Strait				
Fall-run: $P < 0.0001$				
Southern Vancouver Island $P = 0.02$				

81. Nanaimo River	100	42.1	23.7	0.086
82. Cowichan River	100	42.1	26.3	0.100
83. Chemainus River	100	34.2	21.1	0.091
84. Goldstream River	100	44.7	23.7	0.094
Central Vancouver Island $P = 0.04$				
85. Big Qualicum FH	200[a]	55.3	26.3	0.088
86. Little Qualicum	100	47.4	26.3	0.091
87. Puntledge FH	100	44.7	23.7	0.087
Southern south coast $P = 0.28$				
88. Cheakamus River	100	44.7	23.7	0.090
89. Indian Arm	100	44.7	23.7	0.084
Central south coast $P = 0.12$				
90. Mamquam River	100	39.5	26.3	0.083
91. Tzoonie River	100	47.4	23.7	0.092
92. Sliammon FH	100	44.7	23.7	0.092
Mean: wild populations		43.0	24.3	0.090
Mean: hatchery populations		48.2	24.6	0.089
Fraser River				
Fall-run: $P < 0.0006$				
Lower: $P = 0.97$				
93. Alouette River	100	42.1	26.3	0.103
94. Stave River	100	44.7	26.3	0.095
Central: $P < 0.001$				
95. Chilliwack – Vedder FH	100	39.5	26.3	0.095
96. Chehalis at Harrison FH	100	44.7	26.3	0.095
97. Inch Creek FH	100	39.5	23.7	0.091
98. Weaver River	100	39.5	26.3	0.100
99. Chehalis FH	100	50.0	26.3	0.088
100. Harrison River	100	52.6	26.3	0.091
101. Squakum Creek	100	47.4	26.3	0.099
102. Wahleach Creek	100	47.4	26.3	0.098
Mean: wild populations		45.6	26.3	0.098
Mean: hatchery populations		43.4	25.6	0.092
West coast of Vancouver Island				
Fall-run: $P = 0.002$				
103. Nitinat River and FH	380[a]	55.3	21.1	0.086
104. Nahmint River	100	44.7	23.7	0.088
105. Sarita River	127[a]	52.6	23.7	0.092
Mean		50.9	22.8	0.090
Mean of all populations		41.5	26.2	0.098

[a]Pooled collections taken during more than one year. Used to test temporal allele frequency stability at a location.

[b]Hatchery strain that originated from Hood Canal, and hybridized wild populations that were excluded from the gene diversity analysis.

Electrophoresis

We used horizontal starch-gel electrophoresis following the general methods of Aebersold et al. (1987) to assay genetic variation in chum salmon. Locus and allele nomenclature follows Shaklee et al. (1990a). The mobility of alleles follows the recommendations of the coastwide genetics consortium (a group of agencies from the west coast of the United States and Canada that have coordinated and standardized electrophoretic methods and nomenclature for *Oncorhynchus* species). We examined between 36 and 68 loci in any single collection and a total of 90 loci were screened in chum salmon throughout the study area. Allele frequencies at the following 33 loci are presented in Appendix 1 (*mAAT-1**, *sAAT-1,2**, *sAAT-3**, *mAH-1,2**, *mAH-3**, *mAH-4**, *ALAT**, *ESTD-1**, *GPI-A**, *GPI-B1,2**, *G3PDH-2**, *mIDHP-1**, *sIDHP-1**, *sIDHP-2**, *LDH-A1**, *LDH-B1**, *LDH-B2**, *mMDH-3**, *sMDH-A1**, *sMDH-B1,2**, *mMEP-2**, *MPI**, *PEPA**, *PEPB-1**, *PGDH**, *PGM-2**, *sSOD-1**, *TPI-1**, and *TPI-3**). We used the allelic variation at these 33 loci plus 6 additional loci (Appendix 1 footnote) for analyses with the exception of *sAAT-3**, which was not used for estimating the amount of genetic variation, gene diversity, and genetic distances because 20 collections were missing data. When some collections were missing data at *CK-A1** and *PEPA**, we used an allele frequency of 1.0 with a sample size of one fish because the variation at these loci was rare. Inheritance studies have confirmed the genetic basis of many of these loci in chum salmon (May et al. 1975; Beacham et al. 1985; Seeb and Seeb 1986) and other salmonid species (additional references in Shaklee and Phelps 1990; 1992).

The following alleles were pooled prior to analyses: *ALAT*103* with the **105* and **98* with **100*, *sIDHP-2*65* with the other allele of the genotype and the six **65/65* homozygotes were excluded (the *sIDHP-2*65/65* homodimer migrates to the same location on the gel as *sIDHP-1*100/100* homodimer), *sMDH-A2*50* with **100* (the *sMDH-A2*50/50* homodimer migrates to the same location as the *mMDH-A1*100/100* homodimer), *mMEP-2*75* with **100* (the *sMEP-2*75/75* homotetramer migrates to the same location as the *mMEP-1*100/100* homotetramer), and *MPI*95* with the **92* allele.

Alleles at *PEPB-1** were determined by scoring the isozyme variation on both a high pH and a low pH electrophoresis buffer. The *a* allele was scored when the isozyme had the common mobility on both buffers (**100* on the high pH buffer and **-100* on the low pH buffer), the *b* allele was scored when the isozyme had a mobility of **67* on the high pH buffer and **-146* on the low pH buffer, the *c* allele was scored when the isozyme had a mobility of **100* on the high pH buffer and **-126* on the low pH buffer, and the *d* allele was scored when the isozyme had a mobility of **67* on the high pH buffer and **-126* on the low pH buffer.

The loci *mAAT-2**, *ADA-2**, *DIA**, *bGALA-2**, *GAPDH-3**, *GAPDH-5**, *GDA**, *bGLUA**, *sMEP-1**, *PEP-LT**, *PGM-1**, and *PGM-3,4**, were variable, but were not used because of poor resolution, activity, or an uncertain genetic basis of the allozyme patterns. Two other variable loci, *ESTD-2** and *GAPDH-2**, were not used in these analyses because many of the collections were not assayed for these loci. Rare variation (generally less than five variants total) was observed at *sAAT-4**, *ADA-1**, *sAH**, *CK-A2**, *CK-B**, *CK-C1**, *CK-C2**, *FDHG**, *FH**, *G3PDH-3**, *IDDH-1**, *IDDH-2**, *LDH-C**, *ME**, *mMEP-1**, *NTP**, and *PEPD**. No variation was observed at *ADH**, *AK**, *ENO-1**, *ENO-2**, *FBALD-3**, *FBALD-4**, *GAPDH-4**, *GR**, *bGUS**, *mIDHP-2**, *LGL**, *mMDH-1**, *mMDH-2**, *sMEP-2**, *PK-1**, *PK-2**, *PNP**, *mSOD**, and *sSOD-2**. Many of these loci were assayed in a few collections from a geographical region and subsequently dropped from the enzyme screening protocol.

We used the Washington Department of Fish and Wildlife genetic stock identification computer data acquisition and management system to tabulate and error check most of the laboratory data. Manual data recording followed by keypunching was used to collect data for some of the 1987 and 1988 collections. Direct computer entry of the data eliminated the need for keypunching and editing of the genotype data. Laboratory procedures included two important data checking measures to ensure accurate genetic data. The electrophoretic isozyme phenotypes for each fish at each locus were independently interpreted and scored by two individuals, and then the scores were checked by the program for agreement. If the two scores disagreed, an independent third score (agreeing with one of the first two scores) or a supervisor's score was required to resolve all discrepancies before the scores were accepted into the data base. In addition,

numerous loci were scored independently in two tissues or on two different buffer systems. This laboratory protocol served as a check for switches of tissues among fish samples during field sampling, for switches of test tubes or even entire racks of tubes during laboratory analysis, and provided a second, independent set of scores for these loci (Shaklee and Phelps 1990).

Data Analysis

The computer program BIOSYS-1 (Swofford and Selander 1989) was used for calculating allele frequencies, average heterozygosities, percentage of polymorphic loci, average number of alleles per locus, genetic distances, agreement to Hardy–Weinberg expectations, heterogeneity chi-square tests and cluster analysis. Agreement of observed genotypes within each of the 105 populations to Hardy–Weinberg expectations was tested using the chi-square goodness-of-fit test. Only loci that had at least five variant individuals were included. When more than two alleles were observed at a locus, we used the pooling option in BIOSYS-1 that pools the genotypes into three classes (homozygotes for the most common allele, heterozygotes for the most common allele and one of the other alleles, and all other genotypes). We chose this option because the chi-square test values can be inflated in cases where expected frequencies of some classes are low (Sokal and Rohlf 1981). The duplicated isoloci (*sAAT-1,2**, *mAH-1,2**, *GPI-B1,2**, and *sMDH-B1,2** were not tested for agreement with Hardy–Weinberg expectations because of the inability to assign the individual alleles of a genotype to a single locus (Allendorf and Thorgaard 1984). The percentage of polymorphic loci and average heterozygosity between some geographical and run-timing groups were tested with a *t*-test using arcsine-transformed frequencies.

The chi-square test of allelic counts was used to test among-year heterogeneity of collections from the same location and run timing taken in different years, and was used to test between summer-run and fall-run collections within the same or geographically adjacent streams. The null hypothesis was that the collections differed no more than would be expected from taking replicate samples from the same random-mating population. Although the null hypothesis is violated owing to the temporal nature of these collections, Waples (1989b) found that the true α level of tests adjusted by allelic change due to drift, was close to the nominal $\alpha = 0.05$ in most cases. Genetic drift should be a factor in changing allele frequencies only in the Hood Canal and Strait of Juan de Fuca summer-run collections and Thomas Creek, because the remaining locations have spawning populations numbering in the thousands. In addition, sampling of adults exclusively avoids the concerns of results from juvenile collections (Allendorf and Phelps 1981; Waples 1989b). Collections in different years from the same location were pooled in all cases except that the Samish Hatchery collection from 1985 was excluded. Only the 1991 collection from this location was used for population analyses because stock transfers may have been responsible for the significant allele-frequency changes so we did not believe pooling was appropriate.

Relationships among collections were examined using multidimensional scaling, cluster analysis using the unweighted pair-group method with arithmetic averaging (UPGMA), and neighbor-joining (Saitou and Nei 1987) using the genetic chord distance measure of Cavalli-Sforza and Edwards (1967) and the NTSYS-pc software of Rohlf (1992). Neighbor-joining was used to depict the phylogenetic relationships among collections.

The log likelihood ratio test (*G*-test; Sokal and Rohlf 1981) was used to test equality in allele frequencies among all possible pairs of collections. We did these tests to determine if there were any pairs of collections from different fishery management regions that were not significantly different (see Discussion for why these tests were done). Because we were not concerned with the total number of significant tests, we did not modify the significance level to correct for the number of significant tests when multiple tests of the same hypothesis were made. The four isoloci were treated as a single locus for these tests.

A modification of gene-diversity analysis (Chakraborty 1980; Chakraborty et al. 1982) was used to examine hierarchical genetic diversity. The modification consisted of giving equal weight to subgroups within a cell, rather than weighting them according to the number of samples within each subgroup. We chose to analyze the data this way because the number of collections in a geographical area was skewed to the Puget Sound region and the number of subgroups in a cell usually did not reflect the true number of subgroups that may have existed for that cell. This was especially true for the British Columbia collections. Total among-stock gene diversity was partitioned into three levels: diversity within geographical regions within a run timing, diversity among geographical regions within a run timing, and diversity among run timings (refer to Table 1 for the hierarchical structure). Six hatchery and three wild collections from southern Puget Sound, northern Puget Sound, and the Strait of Juan de Fuca were eliminated from the hierarchial gene-diversity analysis because of past stock transfers in which fish from outside the geographical region were used (collection Nos 5, 10, 29, 31, 32, 34, 37, 56, 58; Table 1). We used stock transfer records, the results of the *G*-test analysis, and clustering (dendrograms, and multidimensional scaling) of Cavalli-Sforza and Edwards (1967) chord genetic distances as the basis for elimination. Including these collections would artificially inflate the among-stock within-region diversity estimate.

Results

Distribution of Allelic Variation

Allelic variation at the following 12 loci was common and widespread: *mAAT-1**, *sAAT-1,2**, *sAAT-3**, *mAH-3**, *ALAT**, *G3PDH-2**, *mIDHP-1**, *sIDHP-2**, *mMEP-2**, *MPI**, and *PEPB-1** (Appendix 1). The alleles coding for the common variant isozymes were found in every population examined. The allele variation at the remaining loci used for analysis was rare, generally occurring at a frequency of less than 6% in most collections, and in many cases had a regional distribution (*mAH-1,2**, *mAH-4**, *CK-A1**, *ESTD-1**, *GPI-A**, *GPI-B1,2**, *G3PDH-1**, *sIDHP-1**, *LDH-A1**, *LDH-A2**, *LDH-B1**, *LDH-B2**, *mMDH-3**, *sMDH-A1**, *sMDH-A2**, *sMDH-B1,2**, *PEPA**, *PGDH**, *PGM-2**, *sSOD-1**, *TPI-1**, *TPI-2**, *TPI-3**, and *TPI-4**).

We detected most localized alleles in chum salmon populations, although most were present at low frequencies. The *sAAT-1, 2*84* allele was found only in Nisqually River winter-run chum salmon (21). In Hood Canal summer-run

stocks (42–47), the *sAAT-3*74* allele occurred at frequencies as high as 7%, and the *CK-A1*75* and *CK-A2*105* alleles were rare. The *GPI-A1*105* allele occurred at frequencies as high as 3.4% in the Samish Hatchery (56), but the same or similar allele has been observed in Alaska and in one fish in southern Puget Sound. The *G3PDH-1*-76* allele occurred only in the Pysht River (68), Washington and the Sooke River (69) and Nitinat (103) area on Vancouver Island at 1 or 2%. Another *G3PDH-1** allele, the **-110,* only occurred in the Goldstream River (84), British Columbia. The *sIDHP-2*28* allele was detected only in North Puget Sound, mainly in the Stillaguamish River (48–51) populations. Several other *sIDHP-2** alleles occurred at low frequency in single collections. The *LDH-A1** allele occurred in Washington and Oregon coastal streams, at frequencies as high as 5.5% (Grays River) (79). It appears from photographs that this is a different allele than the **-50* allele that is found at low frequencies in Georgia Strait populations and high frequencies in Alaskan and Asian populations, but we have not confirmed this with allele mobility standards. The *LDH-B1*160* allele has only been observed in the two Columbia River tributary streams and was at a frequency of 5.5% in the Grays River (79). A *sMDH-A1*400* allele was detected only in the two Willapa Bay collections (76, 77). The *PEPB-1*-72* allele was detected in the Lyre River (67) only. *PGM-2*128* alleles occurred only in northern Puget Sound streams (Skagit, Stillaguamish, and Skykomish). The *TPI-1*-50* allele was found mainly in fall-run chum populations in eastern Hood Canal (22–26), although it was found occasionally in western Hood Canal streams. Another TPI variant, *TPI-3*105*, occurred in Deep Creek (66) and Lyre River (67) in the Strait of Juan de Fuca, and was also observed in Bitter Creek (77) in Willapa Bay.

Variation Within Populations

We found slightly more loci out of Hardy–Weinberg equilibrium than what would be expected by chance (80 significant tests out of 1120 total = 7.1% at the $P < 0.05$ level). A significant deficit of heterozygotes occurred 61 times. The pooling of the annual repeat collections prior to Hardy–Weinberg tests may have been partially responsible for the increased number of significant tests. The number of significant tests varied among the 10 loci with common and widespread alleles (*mAAT-1** (12), *sAAT-3** (3), *mAH-3** (3), *ALAT** (6), *G3PDH-2** (7), *mIDHP-1** (9), *sIDHP-2** (13), *mMEP-2** (8), *MPI** (8), and *PEPB-1** (2)). Two populations, Deep Creek (66) and Bitter Creek (77), had three loci; 18 populations had two loci; and 38 had one locus significantly out of equilibrium. An excess of rare homozygotes was the cause for many significant tests.

The mean average heterozygosity (direct count) of all the chum salmon populations based on 38 loci (the 39 loci listed in Appendix 1 excluding *sAAT-3**) was 9.8% and it varied among regions and run-time groups (Table 1). Hood Canal fall-run and southern Puget Sound fall-run and summer-run populations had the highest measures (10.8 and 10.2%). The Strait of Juan de Fuca fall-run, Georgia Strait, and west coast of Vancouver Island populations had the lowest levels (9.0, 9.0, and 8.9%). The Fraser River populations had a significantly higher level of average heterozygosity than the west coast of Vancouver Island populations ($P < 0.025$, $t = 2.39$, df = 11). The only large difference between the direct count and expected equilibrium values was in the northern Puget Sound region where the direct count was 0.5% less.

The percentage of loci that were polymorphic within populations at 1 and 5%, or greater, averaged 41.5 and 26.2%, respectively. These two measures varied significantly among geographical regions and run timings (Table 1). Fall-run chum salmon populations from southern Puget Sound had significantly less variation at the 1% level than fall-run populations from Hood Canal and northern Puget Sound ($P < 0.01$, $t = 2.57$, df = 31; $P < 0.025$, $t = 2.34$, df = 28). The west coast of Vancouver Island populations had the highest mean percentage of loci variable at 1% (50.9%), but the lowest at 5% and were significantly different from Fraser River populations at both measures (at 1%: $P < 0.05$, $t = 1.94$, df = 11; at 5%: $P < 0.001$, $t = 4.94$, df = 11). Hatchery populations were not significantly different from wild populations in Hood Canal, Georgia Strait, and the Fraser River ($t = 0.29$, df = 18; $t = 1.70$, df = 10; $t = 0.72$, df = 8). The Hood Canal summer-run populations had the highest mean level of loci variable at the 5% criterion (30.3%), but one of the lowest mean levels at the 1% criterion (35.1%), and were significantly less variable at the 1% level than Hood Canal wild fall-run populations ($P < 0.001$, $t = 3.90$, df = 22). Summer-run chum from the Strait of Juan de Fuca were also significantly less variable at the 1% level than Hood Canal wild fall-run populations ($P < 0.01$, $t = 2.76$, df = 19), but southern Puget Sound summer-run and fall-run populations were not different from each other at either criterion. Both the Hood Canal and Strait of Juan de Fuca summer-run chum groups have experienced depressed numbers of spawners the last several generations (WDF et al. 1993).

We examined allelic frequency variability from annual repeat collections at 29 locations (Table 1). Populations at five of these locations had significant allelic change at the $P < 0.05$ level. Perry Creek (2) is near an area where nonlocal chum from Hood Canal have been released in the past, however the Swift Creek (1) population in the same inlet was homogeneous over 4 yr. The significant change in Kennedy Creek (12) was due to differences at the loci used for a genetic mark, *sAAT-1,2** and *PGDH** (Seeb et al. 1986; Seeb et al. 1990). The frequencies in the 1985 collection for the **100* allele were 0.885 and 0.990, respectively, for the two loci (which is similar to the frequencies prior to the genetic marking), whereas in the 1991 collection, the frequencies were 0.797 and 0.905, respectively, similar to what was expected for the post-marking equilibrium allele frequency for *sAAT-1,2** and greater than what was expected for *PGDH** (Seeb et al. 1986). Collections from the Samish Hatchery (56) and Thomas Creek (57), both in the Samish River Drainage, had temporal variation. The 1985 Samish Hatchery collection was identified as a mixture of a nonlocal hatchery strain and wild fish, and the 1991 collection had allele frequencies that were more similar to other local stocks. Genetic drift due to small effective population size is a likely explanation for the significant temporal variability in Thomas Creek (57) and in the summer-run Lilliwaup Creek (47) population.

Genetic Differences Among Populations

Significant allelic heterogeneity was found within all run-timing components within geographic regions (Table 1). The likelihood of homogeneity within regions was less than

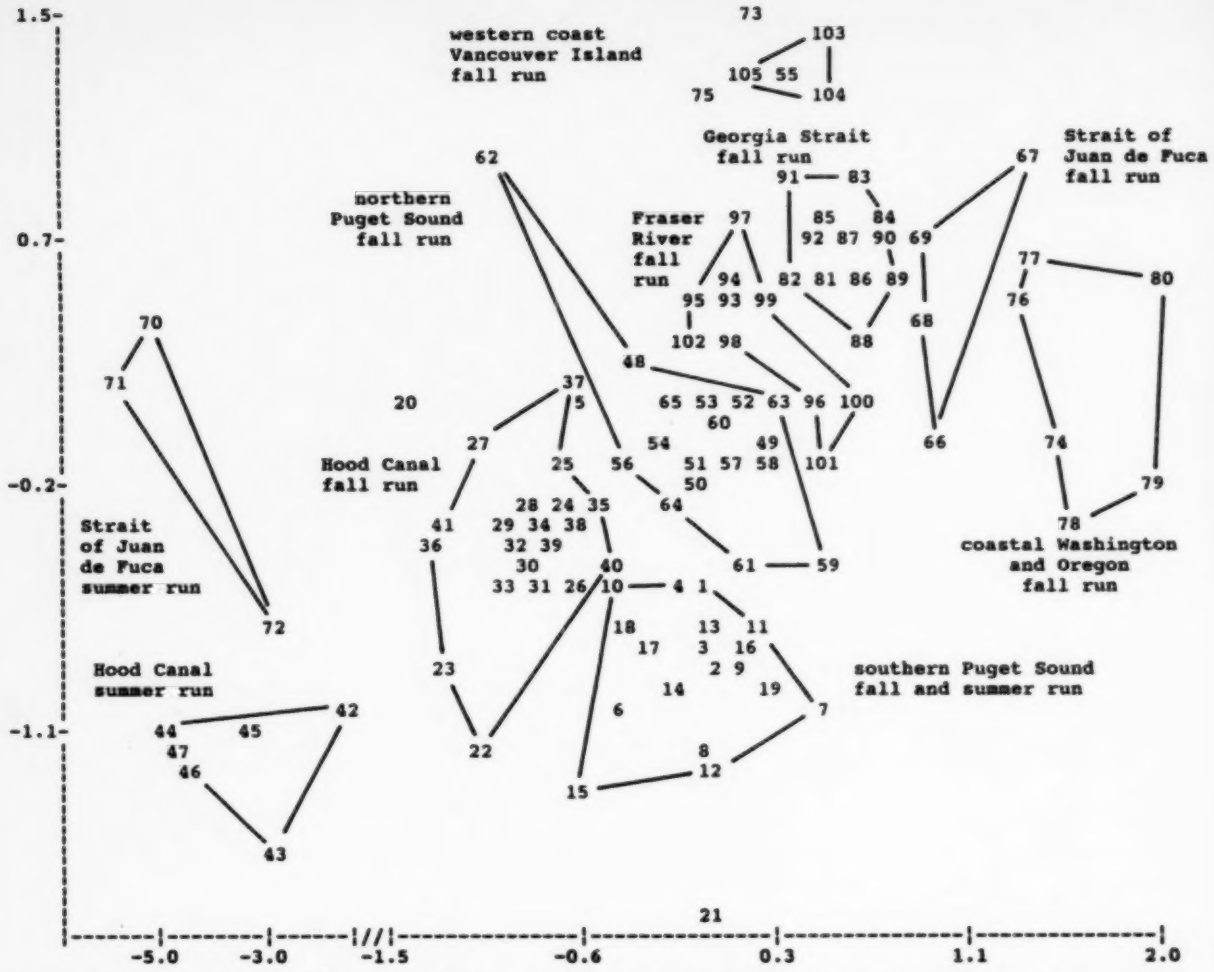

FIG. 2. Two-dimensional representation (from multidimensional scaling) of Cavalli-Sforza and Edwards (1967) chord distance among populations analyzed in this study. The numbers correspond to locations in Table 1 and Fig. 1. The 10 irregular polygons define the major regional and run-timing groups identified in this study except for populations 5, 20, 21, 73, 75 which did not cluster with their respective geographic groups. Population 55 was contained in the west coast of Vancouver Island fall-run polygon but is located in northern Puget Sound.

0.0001, except for the Fraser River and west coast of Vancouver Island regions, which had probabilities of homogeneity of 0.0006 and 0.002, respectively. Of 21 heterogeneity tests within subregions within a run timing, 14 were significant at the $P < 0.05$ level (Table 1).

In geographical regions that contain chum populations with different run timings, significant allelic heterogeneity also occurred among all run-timing groups ($P < 0.0001$). Significant heterogeneity also occurred between all regions and run-timing groups ($P < 0.0001$).

Multidimensional scaling of the Cavalli-Sforza and Edwards chord distance (1967) clustered populations into groups based on geography and run timing (Fig. 2). Stress (a measure of the badness of fit) was 0.19 and is considered only fair (Rohlf 1992). We broke the *X*-axis in this figure because the large distance between the summer-run chum from Hood Canal and the Strait of Juan de Fuca and the other populations obscured the differences among the other populations. The scale on the figure was retained so that the magnitude of the differences can be seen. Neighbor-joining results (not shown) also put the Hood Canal and Strait of Juan de Fuca summer-run chum on a separate branch from all other populations.

In the multidimensional scaling figure only one population, Finney Creek (55) from northern Puget Sound, is within a cluster atypical of its geographic area and genetic origin (west coast of Vancouver Island). This was a small collection ($N = 41$) and may not be a good characterization of this population. In general, the Strait of Juan de Fuca fall-run and coastal Washington and Oregon fall-run chum salmon are more similar to Georgia Strait and the west coast of Vancouver Island populations than to Puget Sound populations. Two populations from the Chehalis River system in Washington, Satsop River (73) and Stevens Creek (75), are depicted closer to the west coast of Vancouver Island populations than other coastal Washington populations. The

Hood Canal fall-run cluster contains several hatchery populations of this gene pool that exist in other geographic regions: Minter Creek (29) and Keta Creek (34) in southern Puget Sound, Tulalip (32) and Bellingham Maritime Hatchery (37) in northern Puget Sound, and lower Elwha (31) in the Strait of Juan de Fuca. The Samish Hatchery (56) had a mixed origin of local and Big Quilcene Hatchery (36) fish and it fell intermediate among other northern Puget Sound and Hood Canal populations.

A dendrogram of Cavalli-Sforza and Edwards (1967) chord distance using UPGMA clustering (Fig. 3) was used as an additional, more conventional method to identify relationships among populations. Major clusters generally contained populations of the same geographic region and run timing. The cophenetic correlation was 0.83 and is considered a good fit (Rohlf 1992). As was seen with the multidimensional scaling, summer-run chum populations from southern Puget Sound cluster with the fall-run populations from this region, instead of with other summer-run populations.

We used the *G*-test (Sokal and Rohlf 1981) (all allele frequency variation at 39 loci) to identify pairs of populations in different regions that were not significantly different (at $P < 0.05$) in their allele frequencies. A total of 38 pairwise comparisons in different regions were not significantly different. The majority of these tests (28) involved the populations with small sample sizes. The Wynoochee River (76) collection with a sample size of 22 was not significantly different from populations in many regions at 15 tests. The Finney Creek (55), Nooksack River (59), and Chuckanut Creek (62) collections from northern Puget Sound had sample sizes of 41, 36, and 35 fish, respectively. Seven nonsignificant pairs involving Finney Creek occurred with Georgia Strait and Fraser River populations. Nooksack River (59) and Chuckanut Creek (62) were not significantly different from Hood Canal populations at four and two pairwise tests, respectively.

Nonsignificant pairwise tests (at $P < 0.05$) involving populations with sample sizes of 50 or greater occurred 10 times. The only nonsignificant test between a United States population and a Canadian population was the Skagit River (52) and the Wahleach River (102) ($P = 0.07$). The Fraser River collection from the Chehalis River hatchery (99) was not different ($P < 0.10$) from three Georgia Strait populations (Nanaimo River (81), Cowichan River (82), and Sliammon Hatchery (92)). The Cowichan River did not differ from the Wahleach River (102) population. Two southern Puget Sound collections, Fennel Creek (5) and Sherwood, Rocky, and Coulter creeks combined (10) were not significantly different involving one and four tests, respectively, with Hood Canal populations. The inclusion of low-frequency allelic variation that adds degrees of freedom, but not much to the *G*-test value, and the treatment of the isoloci as a single locus (for these tests) may have added to the number of nonsignificant tests.

Seven of the streams we studied in Hood Canal and southern Puget Sound have both summer and fall run-timing components (the hatchery strains were developed from local broodstocks). In addition, the summer-run Blackjack Creek population (19) in southern Puget Sound is near other streams that contain naturally spawning fall-run chum (Gorst Creek (15) was used for the test). We found significant differences in allelic counts at variable loci in all tests between run times in each location (Dosewallips River (Nos. 39 and 44) $\chi^2 = 163$, df = 21, $P < 0.001$; Hamma Hamma River (Nos. 38 and 45) $\chi^2 = 80$, df = 19, $P < 0.001$; Lilliwaup River (Nos. 30 and 47) $\chi^2 = 132$, df = 23, $P < 0.001$; Big Quilcene River hatchery (Nos. 36 and 46) $\chi^2 = 228$, df = 20, $P < 0.001$; Sherwood, Rocky, and Coulter creeks combined fall run vs. Sherwood Creek summer run (Nos. 10 and 17) $\chi^2 = 39$, df = 21, $P < 0.01$; Johns Creek hatchery vs. Elson Creek hatchery (the Johns Creek fall-run strain at the Elson Creek hatchery was pooled with other Elson Creek Hatchery collections) (Nos. 16 and 3) $\chi^2 = 63$, df = 20, $P < 0.001$; Sherwood, Rocky, and Coulter creeks combined fall run vs. Coulter Creek hatchery summer run (Nos. 10 and 18) $\chi^2 = 42$, df = 25, $P < 0.018$; Blackjack Creek vs. Gorst Creek (Nos. 19 and 15) $\chi^2 = 47$, df = 20, $P < 0.006$). A greater genetic difference occurred between summer-run and fall-run chum in Hood Canal streams than between the two run-timing components in south Puget Sound. In general, the Hood Canal streams had chi-square values that were twice as large as those at southern Puget Sound locations. Streams containing winter-run chum, Nisqually River (21) and Chambers Creek (20), do not have summer-run or fall-run stocks (the Chambers Creek fall run is extinct) so we could not test for reproductive isolation within a stream for this run timing. We did not sample different run-time components in British Columbia to determine if reproductive isolation between run-timing components exists in this area also.

Hierarchical gene-diversity analysis revealed that 97.17% of the diversity was within populations. Diversity among populations of the same run timing within geographical subregions and among subregions within a region was 0.80 and 0.27% (28.5 and 9.5% of the remaining diversity), respectively. The diversity within a run timing and between run timings was considered two different ways. For the first way, all populations within a run timing (summer, fall, and winter) were clustered together and then the diversity among run timings was calculated. These results were 1.15% for among regions within a run-timing type, and 0.06% among the three run-timing types (40.6 and 21.4% of the remaining diversity). For the second way, all populations within a run timing except the southern Puget Sound summer-run populations were clustered together, then they were clustered with southern Puget Sound fall-run populations, and then the diversity among run timings was calculated. These results were 0.91% among regions within a run timing, 0.05% for southern Puget Sound summer run, and 0.80% for diversity among the three run timings (32.2, 1.6, and 28.2%). We calculated the gene diversity these two ways because of the greater similarity of southern Puget Sound summer-run chum to fall-run chum than to the summer-run chum from Hood Canal and the Strait of Juan de Fuca.

Discussion

Preserving and protecting the fish resources while providing for harvest in fisheries are parts of the mandate of our agency. However, conserving the patterns of genetic variation in natural fish populations is complex (Ryman 1991). An important first step is the knowledge of the allelic distribution and genetic divergence among populations (see Berst and Simon 1981; Ryman 1981; Frankel and Soulé 1981, Soulé 1986, 1987; Ryman and Utter 1987; Whitmore 1990). Integrating knowledge of population structure with

Cavalli-Sforza and Edwards Chord Distance
0.080
0.064
0.048
0.032
0.016

management practices is necessary for developing a genetic conservation plan for a species.

We identified a large amount of genetic structuring in the chum salmon populations in this study area. However, the similarity of chum populations in eastern and western Hood Canal streams, and those in the eastern (mainland side) and western (Vancouver Island) side of Georgia Strait indicates gene flow among populations even though they are on opposite sides of marine waters. These similarities could also be caused by straying from large hatchery programs. Genetic distances between Russian chum salmon populations have been lessened by gene flow from hatchery transplants even though the transplanted fish survived at a reduced rate (Altukhov and Salmenkova 1987, 1990).

The allozyme work of Okazaki (1982) on Japanese chum salmon identified regional clusters of genetically similar populations, but patterns of genetic variation were different on Honshu and Hokkaido. The north–south pattern of heterogeneity on Honshu was not observed on Hokkaido, presumably because of extensive hatchery stocking, but significant heterogeneity was observed on the eastern side of Hokkaido where fewer transplants had been made.

We identified regional clustering of fall-run chum salmon in British Columbia as did Beacham et al. (1985, 1987). However, unlike this previous work, the UPGMA clustering groups in our study did not contain populations from outside the geographic regions. Also in contrast, we identified less average heterozygosity in the west coast of Vancouver Island populations.

In contrast to steelhead, *O. mykiss* (Reisenbichler and Phelps 1989; Reisenbichler et al. 1992), and pink salmon, *O. gorbuscha* (Shaklee et al. 1991), chum salmon had significant heterogeneity within regions and subregions. Chum populations within major river systems were often significantly different. Widespread stocking of the same hatchery strain was proposed as a reason for the lack of stock differentiation in steelhead, but was not considered a likely cause for the results observed in pink salmon. Chum salmon were most like the population structuring observed in chinook salmon (*O. tshawytscha*) (Utter et al. 1989), where run-timing differences were associated with a large component of the diversity.

Concern about the genetic effects of cultured fish on natural fish populations has received increased attention recently (Waples 1991; Hindar et al. 1991). One area of concern is the reduction in the amount of genetic variation in hatchery populations documented in numerous studies (Allendorf and Ryman 1987). We averaged the genetic variability measures within wild and hatchery chum salmon populations by geographical region and run-timing type to determine if genetic variation has been lost in chum salmon hatchery strains in Washington and British Columbia. In Hood Canal fall-run populations, five hatchery populations and 10 wild populations were similar at all three measures (40.5 vs. 41.3%, 26.6 vs. 26.1%, 10.6 vs. 10.5%) for the average percent polymorphic loci at 1, 5%, and average heterozygosity for hatchery and wild populations, respectively. Similar results were observed for most of the other regions and run timings. However, chum salmon at Georgia Strait hatcheries had 16% greater average polymorphic loci at the 1% level than did the average of nine wild populations, perhaps reflecting the mixing of discrete stocks in the hatcheries. In addition, hatchery populations in other regions of Washington that were derived from the Hood Canal strains (Big Quilcene Hatchery (36) and Hood Canal Hatchery (28)) showed no decrease in genetic variation at these three measures. Similar levels of polymorphism in hatchery and wild populations were also found in chinook salmon (Utter et al. 1989).

Another area of concern is the significant change in allele frequencies over time caused by genetic drift and selection in salmon hatcheries (Allendorf and Ryman 1987). These temporal changes in genetic attributes are not desirable when using allele frequency data for mixed-stock fishery analysis (Waples 1989a; Utter and Ryman 1993) and when hatchery strains are used for wild stock recovery and restoration. Only one of the eight hatchery populations we examined, Samish Hatchery, had significant annual changes in allele frequencies. This contrasts with what Waples and Teel (1990) found in Oregon coastal chinook hatcheries where eight of nine hatcheries had significant temporal changes in allele frequencies. Possible reasons for the stability of chum hatchery populations are: (1) most chum salmon hatcheries are fishery production facilities where thousands of adults are spawned each year, and (2) the fry are usually fed for only a month before release, if at all, and do not spend as much time in the hatchery environment as other salmonids.

We determined through hatchery records and genetic analysis that five chum salmon hatcheries located outside of the Hood Canal region contained a strain of chum salmon that was predominately of the Hood Canal gene pool (Minter Creek (29) and Keta Creek (34) in southern Puget Sound, Tulalip (32) and Bellingham Maritime (37) in northern Puget Sound, and Elwha River (31) in the Strait of Juan de Fuca). In addition, the Samish Hatchery (56) appears to have had mixed population of local and Hood Canal origin hatchery fish.

Because the presence of these hatchery strains limits the application of mixed-stock analysis and potentially jeopardizes wild gene pools through interbreeding, several management actions were initiated (cooperatively with Tribal, State, and Federal agencies). The Minter Creek Hatchery (29) and Keta Creek Hatchery (34) strains are being replaced with southern Puget Sound hatchery strains, the Elson Creek strain (3) is being transferred to Minter Creek Hatchery and a hatchery strain derived from the Chico Creek population (14) is replacing the Hood Canal chum at Keta Creek Hatchery. A genetic mark has been applied to the Tulalip Hatchery (32) chum salmon to allow an estimate of their contribution to fisheries and to determine if significant interbreeding with wild populations is occurring. A feasibility study at the Bellingham Maritime Hatchery (37) has been started to determine the success of culturing chum from the Nooksack River. Stocking of chum salmon from the Elwha River Hatchery (31) has ceased and wild spawning aggregations in the river are being examined to determine the extent of introgression. The Samish Hatchery (56) strain is not being stocked outside the drainage. These management actions are an initial step

Fig. 3. UPGMA clustering of Cavalli-Sforza and Edwards (1967) chord distance among chum salmon populations. The numbers correspond to locations in Table 1 and Fig. 1. Acronyms followed by a lowercase "s" are summer-run populations and a "w" are winter-run populations. SPS = southern Puget Sound, HC = Hood Canal, NPS = northern Puget Sound, SJF = Strait of Juan de Fuca, GS = Georgia Strait, FR = Fraser River, WCVI = west coast of Vancouver Island, WAC = Washington coast, ORC = Oregon coast.

toward reducing the threat of stock transfers disrupting natural patterns of chum salmon genetic diversity.

The multidimensional scaling and *G*-test analyses also identified several wild populations outside of Hood Canal that may have had substantial genetic influence from Hood Canal origin hatchery chum salmon. Fennel Creek (5) and the combined fall-run collection from Sherwood, Rocky, and Coulter creeks (10) appear to have some Hood Canal genetic influence. Additional samples from Chuckanut Creek (62) and Nooksack River (59) are necessary to determine if these locations contain admixtures of Hood Canal origin and wild origin gene pools. Identifiable gene flow from past stock transfers of Hood Canal origin chum into other regions was small compared with the magnitude of the transplants. Altukhov and Salmenkova (1990) concluded that transplanting chum salmon into existing populations to increase fish numbers is ineffective.

Both multidimensional scaling and cluster analysis show that Hood Canal and Strait of Juan de Fuca summer-run chum are very distinct from other chum populations. These populations appear to represent a distinct chum salmon lineage. The reduced amount of low-frequency alleles (<1%) in these populations may be natural or the result of recent population bottlenecks and low rates of gene flow. Reproductive isolation between summer-run and fall-run chum salmon preserves an important component of the genetic diversity of this species in the Pacific Northwest. This isolation should be considered in restoration plans and management actions that could increase the potential for inadvertent interbreeding of these two run timings, such as maintaining both summer-run and fall-run broodstocks at a single hatchery, should be discouraged.

Utter and Ryman (1993) listed six requirements for estimating the origin of fish in mixed-stock fisheries: knowledge of which stocks potentially contribute to the fisheries, the existence of genetic differences among stocks, the ability to identify some of these genetic differences, reliable genetic information on potentially contributing stocks, statistical mixture models, and genetic information from a fishery. We have identified allelic variation at 39 loci in chum salmon and have collected allele frequency data for all of the major populations that are likely to be contributing to chum salmon fisheries in Washington State and southern British Columbia. We have determined that geography and run timing have reproductively isolated many of these populations so that measurable genetic differences exist among populations of interest. The results of population clustering techniques such as multidimensional scaling and dendrograms are useful for determining if adequate population structuring is present to produce accurate stock contribution estimates of mixed-stock fisheries, but computer simulations are necessary to determine if sufficient accuracy and precision exists for the management of specific fisheries (Shaklee et al. 1990c; PSC 1992).

The patterns of allele frequency differences among populations, a knowledge of the accuracy and precision of fishery estimates and the temporal stability in stock allele frequencies have allowed the successful application of genetic stock identification in chum salmon fisheries management. The two main areas of application are annual interception estimates for the Pacific Salmon Treaty and inseason harvest estimates of hatchery origin and wild-origin populations in mixed-stock fisheries in Puget Sound. A potential management application is information on migration paths and harvest rates for stock groups through run reconstruction (Starr and Hilborn 1988; Packer 1992). Shaklee et al. (1990b, 1990c) provides more information on chum salmon fishery applications.

In conclusion, chum salmon in Washington and southern British Columbia are subdivided into numerous reproductively isolated populations. Both geography and run timing are important isolating mechanisms. Maintaining these genetic diversity patterns will be important for managing wild chum populations.

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Appendix

Allele frequencies at 33 polymorphic loci for chum salmon from southern British Columbia, Washington, and Oregon[a]. (*N*) = the number of fish assayed at each locus. Alleles are designated by their mobility relative to the most common allele (**100*).

	mAAT-1[1]				*sAAT-1,2*[1]					*sAAT-3*[1]				*mAH-1,2*[1]				*mAH-3*[1]					*mAH-4*[1]				*ALAT*[1]					*GSTD-1*[1]				*GR-A*[1]				*GR-B1,2*[1]					*GDHA-2*[1]		
Location	(N)	-100	-120	-70	(N)	100	120	65	84	(N)	100	80	70	(N)	100	60	(N)	100	134	116	140	(N)	100	120	106	(N)	100	82	106	70	(N)	100	91	(N)	100	105	(N)	100	145	40	(N)	100	90
Southern Puget Sound																																														
Fall-run																																														
Southern																																														
Swift Creek	250	0.000	0.208	0.026	250	0.077	0.123	----	----	102	0.706	0.294	----	250	0.980	0.001	249	0.295	0.705	----	----	250	1.000	----	----	249	0.785	0.169	0.046	----	200	1.000	----	102	1.000	----	103	1.000	----	----	250	0.958	0.042
Perry Creek	346	0.700	0.248	0.052	346	0.031	0.109	----	----	260	0.646	0.354	----	346	1.000	----	345	0.232	0.665	0.003	----	348	1.000	----	----	344	0.776	0.202	0.022	----	346	1.000	----	262	1.000	----	262	1.000	----	----	346	0.946	0.054
Bear Creek FH	349	0.585	0.378	0.037	350	0.076	0.124	----	----	280	0.723	0.277	----	348	1.000	----	348	0.348	0.656	----	----	348	1.000	----	----	348	0.861	0.109	0.032	----	350	1.000	----	329	0.998	0.002	331	1.000	----	----	348	0.931	0.069
Carbon River	148	0.839	0.365	0.007	150	0.576	0.093	----	----	148	0.740	0.260	----	150	1.000	----	149	0.268	0.732	----	----	150	1.000	----	----	150	0.707	0.207	0.007	----	149	1.000	----	150	1.000	----	150	1.000	----	----	150	0.920	0.080
Fennel Creek	84	0.707	0.293	----	100	0.575	0.095	----	----	80	0.750	0.250	----	100	1.000	----	96	0.283	0.737	----	----	100	1.000	----	----	100	0.935	0.140	0.025	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.930	0.070
Skookum Creek	100	0.715	0.275	0.010	100	0.090	0.103	----	----	89	0.621	0.379	----	100	1.000	----	100	0.300	0.700	----	----	100	1.000	----	----	99	0.727	0.269	0.005	----	100	1.000	----	100	1.000	----	100	0.997	----	0.003	100	0.955	0.045
Little Creek	50	0.640	0.310	0.050	50	0.055	0.145	----	----	50	0.800	0.400	----	50	1.000	----	49	0.337	0.663	----	----	50	1.000	----	----	50	0.990	0.120	----	----	50	1.000	----	50	1.000	----	50	1.000	----	----	50	0.920	0.080
Reisdorf Creek	50	0.830	0.280	0.050	50	0.930	0.070	----	----	50	0.520	0.380	----	50	1.000	----	50	0.340	0.660	----	----	50	1.000	----	----	50	0.940	0.130	0.030	----	50	1.000	----	50	1.000	----	50	1.000	----	----	50	0.940	0.060
Upper Skookum Creek	98	0.633	0.332	0.036	99	0.074	0.126	----	----	100	0.550	0.450	----	98	1.000	----	100	0.260	0.720	----	----	99	1.000	----	----	97	0.742	0.222	0.036	----	100	1.000	----	99	0.995	----	0.005	100	0.960	0.060
Sherwood, Rocky & Coulter Cr	198	0.080	0.330	0.009	196	0.067	0.113	----	----	0	----	----	----	198	1.000	----	198	0.311	0.689	----	----	198	1.000	----	----	198	0.936	0.142	0.024	----	198	1.000	----	198	1.000	----	198	1.000	----	----	196	0.969	0.137
Mill Creek	178	0.702	0.273	0.026	178	0.069	0.130	----	----	149	0.685	0.315	----	179	1.000	----	179	0.341	0.656	0.003	----	179	1.000	----	----	179	0.940	0.137	0.014	----	179	1.000	----	179	1.000	----	179	1.000	----	----	178	0.941	0.059
Kennedy Creek	194	0.742	0.211	0.048	200	0.041	0.159	----	----	192	0.667	0.312	----	200	1.000	----	196	0.253	0.747	----	----	200	1.000	----	----	196	0.750	0.214	0.036	----	200	1.000	----	200	1.000	----	200	1.000	----	----	200	0.952	0.047
Goldsborough Creek	200	0.680	0.290	0.030	200	0.069	0.109	----	----	200	0.763	0.237	----	200	1.000	----	200	0.277	0.722	----	----	200	1.000	----	----	199	0.953	0.096	0.040	----	200	1.000	----	200	1.000	----	200	1.000	----	----	197	0.895	0.104
Kitsap Peninsula																																														
Chico Creek	196	0.713	0.283	0.016	196	0.068	0.131	----	----	97	0.722	0.279	----	196	1.000	----	196	0.347	0.651	0.003	----	198	1.000	----	----	196	0.837	0.136	0.036	----	196	1.000	----	196	1.000	----	172	1.000	----	----	196	0.911	0.089
Gorst Creek	100	0.695	0.315	----	100	0.060	0.140	----	----	100	0.675	0.325	----	100	1.000	----	100	0.308	0.696	----	----	100	1.000	----	----	100	0.905	0.126	0.010	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.895	0.105
Summer-run																																														
Southern																																														
Johns Creek	100	0.000	0.400	----	100	0.000	0.100	----	----	99	0.060	0.332	----	100	1.000	----	100	0.305	0.695	----	----	100	1.000	----	----	100	0.745	0.200	0.055	----	100	0.995	0.005	100	1.000	----	100	1.000	----	----	100	0.930	0.070
Shanaxtad Creek	100	0.585	0.425	0.010	100	0.075	0.125	----	----	0	----	----	----	100	1.000	----	99	0.230	0.770	----	----	100	1.000	----	----	100	0.940	0.150	0.010	----	100	1.000	----	100	0.997	0.003	100	0.995	0.005
Coulter Creek FH	100	0.655	0.340	0.005	100	0.060	0.140	----	----	99	0.682	0.308	----	100	1.000	----	94	0.298	0.702	----	----	100	1.000	----	----	100	0.750	0.205	0.035	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.920	0.080
Kitsap Peninsula																																														
Blackjack Creek	100	0.770	0.230	----	100	0.067	0.133	----	----	0	----	----	----	100	1.000	----	99	0.333	0.667	----	----	100	1.000	----	----	100	0.845	0.140	0.015	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.790	0.210
Winter-run																																														
Chambers Creek	100	0.795	0.215	----	100	0.022	0.077	----	----	0	----	----	----	100	1.000	----	99	0.374	0.626	----	----	100	1.000	----	----	100	0.845	0.130	0.025	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.860	0.140
Nisqually River	300	0.713	0.280	0.007	298	0.037	0.081	----	0.002	192	0.750	0.250	----	300	1.000	----	299	0.249	0.751	----	----	300	1.000	----	----	296	0.903	0.160	0.017	----	300	1.000	----	300	1.000	----	300	1.000	----	----	298	0.941	0.059
Hood Canal																																														
Fall-run																																														
Eastern																																														
Big Mission Creek	67	0.739	0.254	0.007	67	0.041	0.059	----	----	65	0.708	0.292	----	67	1.000	----	67	0.313	0.687	----	----	67	1.000	----	----	67	0.821	0.164	0.016	----	67	1.000	----	67	1.000	----	67	1.000	----	----	67	0.873	0.127
Little Mission Creek	51	0.647	0.324	0.029	51	0.072	0.127	----	----	46	0.658	0.344	----	51	1.000	----	47	0.287	0.713	----	----	51	1.000	----	----	51	0.814	0.147	0.039	----	51	1.000	----	51	1.000	----	51	1.000	----	----	51	0.794	0.216
Big Beef Creek	100	0.585	0.415	----	100	0.000	0.100	----	----	98	0.648	0.364	----	0	----	----	99	0.293	0.707	----	----	0	----	----	----	100	0.785	0.185	0.070	----	100	1.000	----	0	----	----	0	----	----	----	100	0.890	0.110
Dewatto River	248	0.575	0.425	----	251	0.000	0.097	----	----	225	0.720	0.280	----	252	0.996	0.002	252	0.325	0.675	----	----	252	0.996	----	0.004	250	0.840	0.132	0.028	----	252	1.000	----	251	1.000	----	251	0.999	0.001	----	252	0.935	0.065
Tahuya River	90	0.689	0.325	0.006	90	0.033	0.106	----	----	79	0.703	0.297	----	90	0.995	0.006	90	0.325	0.675	----	----	90	1.000	----	----	90	0.791	0.169	0.050	----	90	1.000	----	90	1.000	----	90	1.000	----	----	90	0.956	0.144
Western																																														
Enetai FH	100	0.480	0.540	----	100	0.067	0.112	----	----	0	0.750	0.250	----	100	1.000	----	99	0.364	0.636	----	----	100	1.000	----	----	100	0.775	0.210	0.015	----	100	1.000	----	100	1.000	----	100	0.997	0.003	----	100	0.855	0.145
Hood Canal FH	407	0.646	0.365	----	440	0.052	0.108	----	----	196	0.750	0.242	----	449	0.996	0.004	448	0.353	0.647	----	----	449	1.000	----	----	448	0.857	0.108	0.036	----	449	1.000	----	262	1.000	----	262	1.000	----	----	448	0.827	0.173
Minter Creek FH	100	0.705	0.295	----	100	0.063	0.117	----	----	0	----	----	----	100	1.000	----	99	0.374	0.626	----	----	100	1.000	----	----	100	0.870	0.100	0.030	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.830	0.170
Lilliwaup Creek	94	0.691	0.309	0.011	94	0.023	0.077	----	----	0	----	----	----	94	1.000	----	94	0.362	0.638	----	0.011	94	1.000	----	----	91	0.802	0.154	0.044	----	94	1.000	----	94	1.000	----	94	1.000	----	----	94	0.814	0.186
Lower Skokomish FH	98	0.495	0.485	0.010	100	0.037	0.162	----	----	0	----	----	----	100	1.000	----	100	0.360	0.640	----	----	100	1.000	----	----	100	0.795	0.230	0.005	----	100	1.000	----	100	1.000	----	100	0.995	0.005	----	100	0.845	0.155
Tulalip FH	196	0.464	0.536	----	197	0.001	0.099	----	----	96	0.661	0.339	----	197	1.000	----	197	0.330	0.680	----	0.003	196	1.000	----	----	192	0.753	0.229	0.018	----	197	1.000	----	197	1.000	----	197	1.000	----	----	197	0.848	0.152
Wenatchi FH	100	0.480	0.520	----	100	0.063	0.117	----	----	100	0.700	0.300	----	100	1.000	----	100	0.320	0.680	----	----	100	1.000	----	----	97	0.747	0.227	0.026	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.820	0.180
Keta Creek FH	150	0.620	0.380	----	150	0.035	0.105	----	----	51	0.804	0.196	----	150	1.000	----	150	0.313	0.687	----	----	150	1.000	----	----	148	0.864	0.169	0.027	----	150	1.000	----	51	1.000	----	51	1.000	----	----	150	0.950	0.150
McKernan FH	100	0.640	0.360	----	100	0.005	0.095	----	----	0	----	----	----	100	1.000	----	100	0.375	0.625	----	----	100	1.000	----	----	100	0.885	0.120	0.015	----	100	1.000	----	100	1.000	----	100	1.000	----	----	97	0.948	0.124
Bg Quilcene FH	100	0.570	0.430	----	100	0.067	0.112	----	----	99	0.702	0.298	----	100	1.000	----	100	0.310	0.690	----	----	100	1.000	----	----	99	0.742	0.253	0.005	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.945	0.155
Bellingham Mainstme FH	100	0.460	0.485	0.025	100	0.065	0.135	----	----	99	0.657	0.343	----	100	1.000	----	99	0.429	0.571	----	----	100	1.000	----	----	100	0.750	0.240	0.010	----	100	1.000	----	100	0.995	0.005	100	1.000	----	----	100	0.885	0.115
Hamma Hamma River	195	0.569	0.432	----	196	0.091	0.109	----	----	104	0.677	0.321	0.003	196	0.998	0.004	195	0.308	0.692	----	----	196	0.997	----	0.003	195	0.820	0.153	0.027	----	196	1.000	----	196	1.000	----	196	1.000	----	----	194	0.861	0.149
Dosewallips River	105	0.524	0.476	----	105	0.000	0.100	----	----	105	0.710	0.290	----	105	1.000	----	105	0.267	0.643	----	----	105	1.000	----	----	105	0.790	0.186	0.014	----	105	1.000	----	105	1.000	----	105	1.000	----	----	105	0.833	0.167
Skokomish wild																																														
Vance Creek	101	0.644	0.342	0.015	101	0.011	0.089	----	----	100	0.750	0.250	----	101	1.000	----	101	0.302	0.698	----	----	101	1.000	----	----	101	0.807	0.173	0.020	----	101	1.000	----	101	1.000	----	101	1.000	----	----	101	0.902	0.198
NF Skokomish River	111	0.658	0.437	0.005	111	0.003	0.097	----	----	111	0.783	0.207	----	108	0.997	0.003	111	0.374	0.626	----	----	108	1.000	----	----	109	0.771	0.183	0.037	----	111	1.000	----	108	0.995	0.005	108	0.997	0.003	----	111	0.815	0.185
Summer-run																																														
Eastern																																														
Union River	51	0.510	0.490	----	100	0.057	0.042	----	----	0	----	----	----	100	1.000	----	99	0.510	0.490	----	----	100	1.000	----	----	100	0.915	0.080	0.005	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.860	0.140
Western																																														
Duckabush River	124	0.540	0.460	0.004	125	0.052	0.048	----	----	116	0.648	0.343	0.009	125	0.998	0.002	125	0.540	0.462	----	----	125	1.000	----	----	125	0.884	0.110	----	----	125	1.000	----	125	1.000	----	125	1.000	----	----	125	0.944	0.156
Dosewallips River	102	0.505	0.490	0.005	102	0.049	0.051	----	----	102	0.696	0.367	0.005	102	1.000	----	102	0.647	0.353	----	----	102	1.000	----	----	102	0.969	0.132	----	----	102	1.000	----	102	1.000	----	102	1.000	----	----	102	0.885	0.115
Hamma Hamma River	36	0.500	0.500	----	36	0.051	0.049	----	----	30	0.700	0.300	----	36	1.000	----	36	0.569	0.431	----	----	36	1.000	----	----	36	0.969	0.111	----	----	36	1.000	----	36	1.000	----	36	1.000	----	----	36	0.944	0.056
Quilcene Bay & FH	238	0.484	0.506	----	240	0.050	0.050	----	----	237	0.713	0.259	0.027	240	0.998	0.001	239	0.490	0.510	----	----	240	1.000	----	----	239	0.929	0.071	----	----	240	1.000	----	240	1.000	----	240	1.000	----	----	237	0.920	0.080
Lilliwaup Creek	118	0.504	0.496	----	120	0.041	0.059	----	----	99	0.530	0.424	0.046	120	1.000	----	120	0.558	0.442	----	----	120	1.000	----	----	120	0.858	0.137	0.004	----	120	1.000	----	120	1.000	----	120	1.000	----	----	120	0.883	0.117
Northern Puget Sound																																														
Fall-run																																														
Stillaguamish River																																														
Squire Creek	140	0.686	0.332	----	140	0.013	0.057	----	----	140	0.575	0.425	----	140	0.996	0.004	140	0.368	0.614	----	----	139	1.000	----	----	140	0.796	0.171	0.032	----	140	0.996	0.004	140	1.000	----	140	1.000	----	----	140	0.861	0.139
Jim Creek	151	0.642	0.358	----	151	0.034	0.066	----	----	151	0.648	0.348	0.003	148	1.000	----	151	0.344	0.656	----	----	151	1.000	----	----	150	0.760	0.203	0.017	----	151	1.000	----	151	1.000	----	151	1.000	----	----	146	0.887	0.103
Portson Creek	209	0.684	0.313	0.002	211	0.026	0.074	----	----	209	0.616	0.366	----	211	1.000	----	211	0.379	0.618	----	0.002	211	1.000	----	----	210	0.802	0.161	0.017	----	211	0.998	0.002	101	1.000	----	101	1.000	----	----	210	0.876	0.124
N. F. Stillaguamish River	140	0.664	0.332	0.004	140	0.005	0.095	----	----	140	0.629	0.371	----	140	1.000	----	140	0.364	0.646	----	----	139	1.000	----	----	139	0.813	0.180	0.007	----	140	1.000	----	140	1.000	----	140	1.000	----	----	139	0.888	0.112
Skagit River																																														
Skagit River	100	0.690	0.310	----	100	0.070	0.130	----	----	99	0.694	0.306	----	100	1.000	----	100	0.325	0.675	----	----	100	1.000	----	----	99	0.788	0.212	----	----	100	1.000	----	100	1.000	----	100	0.997	0.003	----	100	0.890	0.110
Baker Creek	98	0.587	0.403	----	98	0.016	0.084	----	----	98	0.684	0.316	----	98	1.000	----	99	0.398	0.602	----	----	98	1.000	----	----	98	0.898	0.193	0.010	----	98	1.000	----	3	1.000	----	98	0.995	0.005	----	98	0.883	0.117
Dan Creek	151	0.659	0.341	----	153	0.010	0.062	----	----	148	0.652	0.348	----	153	0.998	0.002	153	0.373	0.627	----	----	153	1.000	----	----	153	0.779	0.193	0.028	----	153	1.000	----	153	0.997	0.003	153	1.000	----	----	151	0.841	0.159
Finney Creek	41	0.659	0.341	----	41	0.070	0.122	----	----	39	0.692	0.308	----	41	1.000	----	41	0.402	0.598	----	----	41	1.000	----	----	41	0.890	0.073	0.012	0.024	41	1.000	----	41	1.000	----	41	1.000	----	----	40	0.987	0.012

Appendix (*Continued*)

Location	mAAT-1*				sAAT-1,2*					sAAT-3*				mAH-1,2*				mAH-3*				mAH-4*				ALAT*					ISTD-1*				GR-A*				GR-B1,2*				G3PDH-2*		
	(S)	-100	-120	-70	(S)	100	120	65	84	(S)	100	80	70	(S)	100	80	(S)	100	120	115	140	(S)	100	130	105	(S)	100	82	156	70	(S)	100	81	(S)	100	106	46	(S)	100	80
Gamah River																																												
Gamah FH	99	0.068	0.424	0.020	100	0.052	0.147	----	----	100	0.015	0.385	----	99	1.000	----	100	0.365	0.635	----	----	100	1.000	----	----	100	0.095	0.115	----	----	100	1.000	----	100	0.890	0.010	100	1.000	----	----	100	0.950	0.100
Thomas Creek	97	0.577	0.423	----	101	0.064	0.136	----	----	101	0.044	0.356	----	102	0.935	0.065	100	0.360	0.650	----	----	100	1.000	----	----	98	0.962	0.128	0.010	----	102	1.000	----	100	0.970	0.030	99	1.000	----	----	98	0.937	0.062
Bob Smith Creek	95	0.050	0.421	0.021	100	0.072	0.127	----	----	95	0.015	0.384	----	100	0.997	0.003	99	0.590	0.052	----	----	100	0.995	----	0.005	100	0.905	0.190	0.005	----	100	1.000	----	100	0.890	0.005	100	1.000	----	----	100	0.910	0.090
Nooksack River																																												
Nooksack River	30	0.730	0.250	0.014	30	0.944	0.056	----	----	34	0.059	0.441	----	36	1.000	----	36	0.361	0.639	----	----	36	1.000	----	----	36	0.033	0.125	0.014	0.039	36	1.000	----	36	1.000	----	36	1.000	----	----	35	0.900	0.100
Kendall FH	199	0.991	0.317	0.003	200	0.993	0.106	----	----	195	0.994	0.416	----	200	1.000	----	177	0.331	0.669	----	----	199	1.000	----	----	200	0.795	0.197	0.007	----	200	1.000	----	101	1.000	----	101	0.997	0.003	----	200	0.917	0.082
Maple Creek	100	0.765	0.235	----	100	0.870	0.130	----	----	0	----	----	----	100	1.000	----	100	0.275	0.725	----	----	100	1.000	----	----	100	0.935	0.160	0.005	----	100	1.000	----	0	----	----	0	----	----	----	100	0.925	0.075
Bellingham Bay																																												
Chuckanut Creek	35	0.671	0.429	----	35	0.829	0.072	----	----	35	0.071	0.329	----	35	0.993	0.007	36	0.371	0.629	----	----	35	0.986	----	0.014	34	0.997	0.068	0.015	----	36	1.000	----	36	1.000	----	36	1.000	----	----	35	0.943	0.157
Skykomish River																																												
Bolsholhouse Slough	99	0.015	0.360	0.005	99	0.949	0.052	----	----	99	0.808	0.396	----	95	1.000	----	96	0.292	0.709	----	----	99	1.000	----	----	99	0.007	0.197	0.005	----	99	1.000	----	99	1.000	----	99	1.000	----	----	99	0.917	0.083
Wallace River	144	0.015	0.385	----	144	0.936	0.063	0.002	----	143	0.033	0.367	----	144	0.993	0.007	144	0.323	0.674	0.003	----	144	0.997	0.003	----	144	0.930	0.170	0.003	----	144	1.000	----	144	1.000	----	0.002	144	0.990	----	0.002	144	0.990	0.132
Skykomish River	200	0.012	0.387	----	200	0.936	0.062	0.001	----	199	0.721	0.279	----	200	0.999	0.001	199	0.329	0.669	0.006	----	199	0.997	----	0.003	200	0.902	0.177	0.020	----	200	1.000	----	200	1.000	----	199	1.000	----	----	199	0.992	0.108
Strait of Juan de Fuca																																												
Fall-run																																												
Deep Creek	100	0.035	0.305	----	100	0.997	0.112	----	----	99	0.036	0.464	----	100	0.997	0.003	100	0.255	0.745	----	----	100	1.000	----	----	100	0.750	0.230	0.020	----	99	0.995	0.005	100	1.000	----	100	1.000	----	----	100	0.965	0.035
Lyre River	99	0.997	0.330	0.005	100	0.925	0.075	----	----	99	0.072	0.429	----	100	0.980	0.020	100	0.325	0.675	----	----	100	1.000	----	----	99	0.888	0.112	----	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.975	0.025
Pysht River	251	0.721	0.279	----	205	0.917	0.083	----	----	211	0.059	0.341	----	254	0.998	0.002	254	0.248	0.754	----	----	205	0.999	0.002	----	205	0.890	0.108	0.004	----	205	0.998	0.002	249	1.000	----	191	0.997	0.003	----	205	0.967	0.033
Sooke River	99	0.048	0.343	0.010	100	0.903	0.117	----	----	0	----	----	----	100	1.000	----	73	0.430	0.541	0.014	0.007	100	1.000	----	----	100	0.950	0.150	----	----	100	1.000	----	0	----	----	0	----	----	----	100	0.935	0.075
Summer-run																																												
Salmon Creek	50	0.510	0.480	----	50	0.950	0.150	----	----	50	0.340	0.660	----	50	1.000	----	50	0.530	0.470	----	----	50	1.000	----	----	50	0.830	0.190	----	----	50	1.000	----	50	1.000	----	50	1.000	----	----	50	0.990	0.010
Snow Creek	50	0.570	0.430	----	50	0.995	0.105	----	----	50	0.370	0.630	----	50	1.000	----	50	0.700	0.300	----	----	50	1.000	----	----	50	0.980	0.120	----	----	50	1.000	----	50	1.000	----	50	1.000	----	----	50	0.930	0.070
Jimmycomelately Creek	100	0.990	0.305	0.015	100	0.997	0.193	----	----	3	0.167	0.833	----	100	1.000	----	100	0.990	0.360	----	----	100	1.000	----	----	100	0.995	0.155	----	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.975	0.025
Fraser and Columbia River																																												
Fall-run																																												
Chehalis River																																												
Salmon River	100	0.525	0.450	0.025	100	0.935	0.065	----	----	100	0.590	0.410	----	100	1.000	----	100	0.350	0.650	----	----	100	1.000	----	----	100	0.895	0.095	0.010	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.920	0.080
Wynoochee River	22	0.432	0.545	0.023	22	0.943	0.057	----	----	22	0.636	0.364	----	22	1.000	----	22	0.364	0.636	----	----	22	1.000	----	----	22	0.773	0.205	0.023	----	22	1.000	----	22	1.000	----	22	1.000	----	----	22	0.959	0.041
Stevens Creek	100	0.615	0.380	0.005	100	0.935	0.065	----	----	100	0.570	0.430	----	100	1.000	----	98	0.443	0.557	----	----	100	1.000	----	----	99	0.919	0.102	0.020	----	100	1.000	----	100	1.000	----	100	1.000	----	----	98	0.937	0.062
Willapa Bay																																												
Elsworth Creek	100	0.000	0.440	----	100	0.935	0.065	----	----	100	0.425	0.575	----	100	1.000	----	100	0.405	0.595	----	----	100	1.000	----	----	100	0.795	0.215	----	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.950	0.100
Bear Creek	100	0.620	0.375	0.005	100	0.917	0.082	----	----	99	0.485	0.535	----	100	0.997	0.003	99	0.333	0.667	----	----	100	1.000	----	----	100	0.830	0.155	0.015	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.940	0.060
Columbia River																																												
Hamilton Creek	100	0.715	0.280	0.025	100	0.957	0.042	----	----	100	0.725	0.275	----	100	0.995	0.005	99	0.419	0.581	----	----	100	1.000	----	----	99	0.798	0.102	0.010	----	99	1.000	----	99	1.000	----	99	1.000	----	----	99	0.970	0.030
Grays River	100	0.560	0.440	----	100	0.925	0.075	----	----	100	0.615	0.385	----	100	0.997	0.003	100	0.260	0.720	----	----	100	1.000	----	----	100	0.795	0.205	----	----	100	1.000	----	100	1.000	----	100	1.000	----	----	99	0.955	0.045
Coal Creek																																												
Coal Creek	99	0.002	0.372	0.025	97	0.010	0.090	----	----	99	0.501	0.499	----	99	0.987	0.013	99	0.364	0.646	----	----	99	1.000	----	----	99	0.750	0.250	----	----	99	1.000	----	99	1.000	----	97	1.000	----	----	99	0.959	0.041
Georgia Strait																																												
Fall-run																																												
Southern Vancouver Island																																												
Nanaimo River	99	0.967	0.333	----	100	0.902	0.095	0.003	----	0	----	----	----	100	1.000	----	98	0.261	0.710	----	----	100	1.000	----	----	100	0.760	0.235	0.010	0.005	100	0.995	0.005	0	----	----	0	----	----	----	100	0.970	0.030
Cowichan River	100	0.630	0.370	----	100	0.995	0.105	----	----	0	----	----	----	100	1.000	----	99	0.348	0.652	----	----	100	1.000	----	----	100	0.740	0.245	0.010	0.005	100	1.000	----	0	----	----	0	----	----	----	100	0.930	0.070
Chemainus River	99	0.002	0.319	----	100	0.875	0.122	0.003	----	0	----	----	----	100	1.000	----	100	0.336	0.666	----	----	100	1.000	----	----	100	0.790	0.210	----	----	100	0.995	0.005	0	----	----	0	----	----	----	100	0.960	0.040
Goldstream River	100	0.595	0.405	----	100	0.997	0.112	----	----	99	0.077	0.323	----	92	1.000	----	92	0.348	0.652	----	----	92	1.000	----	----	100	0.960	0.195	----	0.005	100	0.990	0.010	100	1.000	----	100	1.000	----	----	99	0.960	0.040
Central Vancouver Island																																												
Big Qualicum FH	199	0.952	0.348	0.003	200	0.911	0.088	----	----	199	0.781	0.220	----	200	1.000	----	200	0.207	0.702	----	----	200	0.992	0.005	0.002	200	0.907	0.182	----	----	200	0.995	0.005	199	1.000	----	200	1.000	----	----	200	0.945	0.055
Little Qualicum	100	0.590	0.410	----	100	0.883	0.117	----	----	97	0.598	0.412	----	100	0.997	0.003	100	0.260	0.720	----	----	100	0.995	0.005	----	100	0.795	0.210	0.005	----	100	0.990	0.010	100	1.000	----	100	1.000	----	----	99	0.949	0.051
Puntledge FH	100	0.665	0.330	0.005	100	0.907	0.093	----	----	99	0.692	0.310	----	97	1.000	----	79	0.291	0.709	----	----	97	1.000	----	----	100	0.600	0.195	0.010	0.005	100	0.980	0.020	70	1.000	----	70	1.000	----	----	100	0.960	0.040
Southern south coast																																												
Cheakamus River	99	0.011	0.389	----	100	0.917	0.082	----	----	0	----	----	----	100	1.000	----	99	0.364	0.636	----	----	100	0.995	0.005	----	99	0.600	0.102	0.010	----	100	1.000	----	0	----	----	0	----	----	----	100	0.935	0.065
Indian Arm	100	0.640	0.355	0.005	100	0.915	0.085	----	----	0	----	----	----	100	1.000	----	98	0.362	0.648	----	----	99	0.995	0.015	----	98	0.721	0.199	0.010	----	100	1.000	----	0	----	----	0	----	----	----	100	0.970	0.030
Central south coast																																												
Marquam River	100	0.620	0.375	0.005	100	0.893	0.117	----	----	99	0.596	0.404	----	100	0.995	0.005	100	0.365	0.635	----	----	100	0.995	0.005	----	100	0.870	0.125	----	0.005	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.935	0.065
Tenmile River	100	0.715	0.285	----	100	0.900	0.100	----	----	100	0.660	0.340	----	100	0.997	0.003	99	0.293	0.707	----	----	100	0.980	0.020	----	100	0.795	0.195	0.010	----	100	0.995	0.005	100	1.000	----	100	1.000	----	----	98	0.962	0.010
Stamman FH	100	0.050	0.350	----	100	0.907	0.093	----	----	92	0.603	0.397	----	99	1.000	----	94	0.303	0.697	----	----	97	0.995	0.005	----	100	0.620	0.180	----	----	100	1.000	----	100	0.995	0.005	100	1.000	----	----	100	0.950	0.040
Fraser River																																												
Fall-run																																												
Lower																																												
Alouette River	100	0.640	0.365	0.005	100	0.935	0.170	----	----	0	----	----	----	100	1.000	----	95	0.332	0.669	----	----	100	1.000	----	----	100	0.795	0.200	0.005	----	100	1.000	----	0	----	----	0	----	----	----	100	0.905	0.095
Stave River	99	0.677	0.323	----	100	0.845	0.155	----	----	0	----	----	----	100	1.000	----	63	0.373	0.627	----	----	100	1.000	----	----	100	0.700	0.175	0.045	----	100	1.000	----	0	----	----	0	----	----	----	97	0.923	0.077
Central																																												
Chilliwack - Vedder FH	100	0.005	0.395	----	100	0.895	0.133	0.003	----	100	0.660	0.340	----	52	1.000	----	100	0.340	0.660	----	----	100	1.000	----	----	100	0.600	0.155	0.045	----	100	0.990	0.010	100	1.000	----	----	----	----	----	100	0.910	0.090
Chehalis @ Harrison FH	99	0.007	0.308	0.005	100	0.905	0.195	----	----	97	0.589	0.412	----	100	1.000	----	100	0.305	0.695	----	----	100	1.000	----	----	100	0.800	0.165	0.035	----	100	1.000	----	100	1.000	----	100	0.997	----	0.003	100	0.915	0.085
Inch Creek FH	100	0.705	0.295	----	100	0.960	0.140	----	----	100	0.035	0.365	----	100	1.000	----	100	0.335	0.665	----	----	100	1.000	----	----	100	0.725	0.220	0.055	----	100	1.000	----	100	1.000	----	100	1.000	----	----	100	0.955	0.045
Weaver River	100	0.710	0.290	----	100	0.852	0.148	----	----	0	----	----	----	100	1.000	----	76	0.373	0.627	----	----	100	1.000	----	----	100	0.720	0.230	0.050	----	100	1.000	----	0	----	----	0	----	----	----	100	0.935	0.065
Chehalis FH	99	0.994	0.316	----	99	0.975	0.125	----	----	100	0.660	0.340	----	100	0.997	0.003	100	0.305	0.695	----	----	100	1.000	----	----	100	0.700	0.195	0.105	----	100	1.000	----	100	1.000	----	100	1.000	----	----	99	0.939	0.061
Harrison River	100	0.690	0.340	----	100	0.945	0.155	----	----	99	0.677	0.323	----	100	0.990	0.010	100	0.405	0.595	----	----	100	1.000	----	----	99	0.770	0.153	0.077	----	100	1.000	----	100	0.995	----	99	1.000	----	----	99	0.934	0.066
Squakum Creek	99	0.737	0.263	----	96	0.990	0.100	----	----	100	0.710	0.290	----	100	0.997	0.003	100	0.370	0.630	----	----	100	1.000	----	----	100	0.705	0.195	0.060	----	100	1.000	----	100	1.000	----	100	0.997	----	0.000	100	0.915	0.085
Wahleach Creek	99	0.007	0.313	----	100	0.862	0.137	----	----	0	----	----	----	100	1.000	----	99	0.417	0.583	----	----	100	1.000	----	----	99	0.737	0.247	0.015	----	100	1.000	----	0	----	----	0	----	----	----	100	0.935	0.065
West Coast Vancouver Island																																												
Fall-run																																												
Nitinat River and FH	370	0.726	0.272	----	370	0.932	0.070	----	----	200	0.477	0.515	0.007	370	0.995	0.002	207	0.210	0.790	----	----	370	1.000	----	----	370	0.821	0.166	0.001	----	370	0.988	0.012	201	1.000	----	201	0.999	0.001	----	370	0.959	0.041
Nahmint River	98	0.760	0.245	0.005	100	0.913	0.088	----	----	100	0.470	0.530	----	100	0.997	0.012	100	0.305	0.695	----	----	100	1.000	----	----	100	0.795	0.205	----	----	0	----	----	0	----	----	0	----	----	----	100	0.965	0.035
Sarita River	126	0.671	0.329	----	127	0.910	0.090	----	----	123	0.459	0.541	----	101	0.995	0.010	127	0.207	0.713	----	----	101	0.995	----	0.005	127	0.843	0.157	----	----	127	0.990	0.004	101	1.000	----	101	0.997	0.003	----	92	0.951	0.049

Appendix (*Continued*)

Location	mDHP-1*					sDHP-1*					mDHP-2*							LDH-A1*			LDH-B1*				LDH-B2*			mMDH-3*				sMDH-A1*			
	(N)	100	60	140	30	(N)	100	70	130	112	(N)	100	26	86	35	110	28	(N)	-100	50	(N)	100	100	300	(N)	100	120	115	(N)	-100	-50	-120	(N)	100	300	400	10
Southern Puget Sound																																					
Fall-run																																					
Southern																																					
Skerk Creek	250	0.812	0.188	----	----	250	0.996	0.004	----	----	250	0.460	0.326	0.168	0.068	----	----	250	1.000	----	250	1.000	----	----	250	1.000	----	----	250	1.000	----	----	250	0.968	0.032	----	----
Perry Creek	348	0.612	0.188	----	----	348	1.000	----	----	----	348	0.485	0.322	0.168	0.046	----	----	348	1.000	----	348	1.000	----	----	348	1.000	----	----	348	1.000	----	----	348	0.957	0.043	----	----
Skagon Creek FH	350	0.839	0.161	----	----	350	1.000	----	----	----	350	0.446	0.297	0.214	0.043	----	----	350	1.000	----	350	1.000	----	----	350	1.000	----	----	350	1.000	----	----	350	0.964	0.036	----	----
Carlsun Rusk	150	0.840	0.160	----	----	150	0.997	0.003	----	----	150	0.529	0.290	0.093	0.087	----	0.007	150	1.000	----	150	1.000	----	----	150	0.997	0.003	----	150	1.000	----	----	150	0.917	0.083	----	----
Fennel Creek	100	0.740	0.260	----	----	100	0.995	0.005	----	----	100	0.530	0.265	0.065	0.146	----	0.006	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.015	----	----
Skookum Creek	100	0.715	0.285	----	----	100	1.000	----	----	----	100	0.450	0.310	0.125	0.115	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.940	0.060	----	----
Little Creek	50	0.960	0.160	----	----	50	1.000	----	----	----	50	0.480	0.380	0.120	0.020	----	----	50	1.000	----	50	1.000	----	----	50	1.000	----	----	50	1.000	----	----	20	1.000	----	----	----
Reisdorf Creek	50	0.770	0.230	----	----	50	1.000	----	----	----	49	0.449	0.306	0.214	0.031	----	----	50	1.000	----	50	1.000	----	----	50	1.000	----	----	50	1.000	----	----	50	0.970	0.030	----	----
Upper Skookum Creek	100	0.800	0.200	----	----	100	1.000	----	----	----	100	0.390	0.350	0.140	0.110	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	----
Sherwood, Rocky & Coulter Cr	108	0.731	0.269	----	----	105	0.990	0.010	----	----	104	0.577	0.215	0.135	0.067	0.005	----	106	1.000	----	108	1.000	----	----	108	1.000	----	----	108	1.000	----	----	108	0.958	0.038	0.005	----
Mill Creek	179	0.858	0.142	----	----	179	0.997	0.003	----	----	179	0.463	0.346	0.126	0.042	----	----	179	1.000	----	179	1.000	----	----	179	0.994	0.003	0.003	179	1.000	----	----	179	0.975	0.025	----	----
Kennedy Creek	200	0.795	0.205	----	----	200	1.000	----	----	----	200	0.467	0.302	0.170	0.070	----	----	200	1.000	----	200	1.000	----	----	200	1.000	----	----	121	0.975	0.025	----	----	----	----	----	----
Goldsborough Creek	200	0.860	0.140	----	----	200	1.000	----	----	----	200	0.536	0.290	0.142	0.032	----	----	200	1.000	----	200	1.000	----	----	200	1.000	----	----	200	1.000	----	----	196	0.980	0.020	----	----
Kitsap Peninsula																																					
Chico Creek	186	0.783	0.217	----	----	185	1.000	----	----	----	194	0.464	0.394	0.111	0.041	----	----	186	1.000	----	196	1.000	----	----	186	1.000	----	----	196	1.000	----	----	196	0.952	0.048	----	----
Sorel Creek	100	0.740	0.260	----	----	100	1.000	----	----	----	100	0.410	0.335	0.215	0.045	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.915	0.085	----	----
Summer-run																																					
Southern																																					
Johns Creek	100	0.770	0.230	----	----	100	0.995	0.005	----	----	100	0.535	0.295	0.170	0.010	----	----	100	0.995	0.005	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.970	0.030	----	----
Sherwood Creek	100	0.790	0.210	----	----	100	1.000	----	----	----	100	0.570	0.250	0.120	0.055	----	0.005	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.935	0.060	0.005	----
Coulter Creek FH	100	0.720	0.280	----	----	76	1.000	----	----	----	100	0.548	0.330	0.110	0.015	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.955	0.045	----	----
Kitsap Peninsula																																					
Seabeck Creek	100	0.870	0.130	----	----	100	1.000	----	----	----	99	0.449	0.429	0.106	0.015	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.995	0.005	----	----
Winter-run																																					
Chambers Creek	100	0.840	0.160	----	----	100	0.995	0.005	----	----	97	0.531	0.170	0.103	0.166	0.005	0.005	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.970	0.030	----	----
Nisqually River	299	0.901	0.099	----	----	299	0.998	0.002	----	----	299	0.597	0.164	0.144	0.102	----	0.003	300	1.000	----	300	1.000	----	----	300	1.000	----	----	300	1.000	----	----	299	0.970	0.030	----	----
Hood Canal																																					
Fall-run																																					
Eastern																																					
Big Mission Creek	67	0.853	0.343	----	----	67	0.993	0.007	----	----	67	0.537	0.276	0.119	0.067	----	----	67	1.000	----	67	1.000	----	----	67	1.000	----	----	67	1.000	----	----	67	0.940	0.060	----	----
Little Mission Creek	51	0.735	0.265	----	----	51	0.980	0.020	----	----	51	0.520	0.255	0.088	0.137	----	----	51	1.000	----	51	1.000	----	----	51	1.000	----	----	51	1.000	----	----	51	0.931	0.069	----	----
Big Beef Creek	99	0.747	0.253	----	----	100	0.995	0.005	----	----	100	0.556	0.290	0.100	0.110	0.005	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.975	0.025	----	----
Dewatto River	261	0.771	0.229	----	----	252	0.966	0.034	----	----	262	0.534	0.272	0.077	0.115	0.002	----	252	1.000	----	252	1.000	----	----	252	1.000	----	----	252	1.000	----	----	210	0.972	0.029	----	----
Tahuya River	80	0.781	0.219	----	----	80	0.981	0.019	----	----	79	0.570	0.222	0.127	0.082	----	----	80	0.994	0.006	80	1.000	----	----	80	1.000	----	----	80	1.000	----	----	61	0.975	0.025	----	----
Western																																					
Dnetsi FH	100	0.765	0.235	----	----	100	0.965	0.035	----	----	100	0.525	0.265	0.090	0.110	0.010	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.925	0.075	----	----
Hood Canal FH	449	0.690	0.310	----	----	449	0.988	0.012	----	----	449	0.521	0.229	0.092	0.056	----	0.001	449	1.000	----	449	1.000	----	----	449	1.000	----	----	449	1.000	----	----	449	0.987	0.013	----	----
Minter Creek FH	100	0.665	0.335	----	----	100	0.965	0.010	0.005	----	99	0.586	0.237	0.081	0.096	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.990	0.010	----	----
Lilliwaup Creek	94	0.676	0.324	----	----	94	0.904	0.016	----	----	93	0.554	0.237	0.108	0.087	----	0.005	94	1.000	----	94	1.000	----	----	94	1.000	----	----	94	1.000	----	----	94	0.979	0.021	----	----
Lower Skokomish FH	100	0.715	0.285	----	----	100	0.995	0.005	----	----	100	0.575	0.225	0.120	0.080	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.960	0.040	----	----
Tulalip FH	196	0.694	0.306	----	----	197	0.985	0.015	----	----	196	0.490	0.265	0.135	0.110	----	----	197	1.000	----	197	1.000	----	----	197	1.000	----	----	197	1.000	----	----	197	0.977	0.023	----	----
Wallace FH	100	0.685	0.315	----	----	100	0.990	0.010	----	----	100	0.465	0.295	0.145	0.105	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.975	0.025	----	----
Kalis Creek FH	150	0.693	0.307	----	----	150	0.990	0.010	----	----	149	0.611	0.201	0.087	0.091	----	----	150	1.000	----	150	1.000	----	----	150	1.000	----	----	148	0.970	0.030	----	----	----	----	----	----
McKernan FH	100	0.710	0.290	----	----	100	0.985	0.015	----	----	100	0.600	0.260	0.070	0.080	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	----	0.005	100	0.970	0.030	----	----
Big Quilcene FH	100	0.670	0.330	----	----	100	0.965	0.035	----	----	100	0.440	0.275	0.125	0.160	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	0	----	----	----	100	0.940	0.060	----	----
Bellingham Maritime FH	100	0.935	0.165	----	----	100	0.985	0.015	----	----	100	0.595	0.215	0.100	0.085	----	0.005	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	99	0.975	0.025	----	----
Hamma Hamma River	186	0.731	0.269	----	----	186	0.989	0.011	----	----	186	0.570	0.226	0.097	0.109	----	----	186	1.000	----	186	1.000	----	----	186	1.000	----	----	186	1.000	----	----	186	0.976	0.024	----	----
Dosewallips River	105	0.705	0.295	----	----	105	0.990	0.010	----	----	104	0.563	0.231	0.115	0.087	----	----	105	1.000	----	105	1.000	----	----	105	1.000	----	----	105	1.000	----	----	104	0.967	0.043	----	----
Skokomish wild																																					
Vance Creek	101	0.723	0.277	----	----	101	1.000	----	----	----	101	0.475	0.361	0.078	0.084	----	----	101	1.000	----	101	1.000	----	----	101	1.000	----	----	101	1.000	----	----	101	0.990	0.010	----	----
NF Skokomish River	111	0.644	0.356	----	----	111	0.991	0.009	----	----	111	0.569	0.264	0.054	0.095	----	----	111	0.995	0.005	111	1.000	----	----	111	1.000	----	----	111	1.000	----	----	111	0.982	0.018	----	----
Summer run																																					
Eastern																																					
Union River	100	0.840	0.160	----	----	21	1.000	----	----	----	100	0.520	0.305	0.115	0.060	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	98	0.939	0.061	----	----
Western																																					
Quilcenum River	126	0.840	0.160	----	----	125	0.988	0.012	----	----	124	0.605	0.262	0.093	0.040	----	----	126	1.000	----	126	1.000	----	----	125	1.000	----	----	124	0.947	0.153	----	----	----	----	----	----
Dosewallips River	101	0.866	0.134	----	----	102	0.995	0.005	----	----	102	0.590	0.336	0.054	0.010	----	----	102	1.000	----	102	1.000	----	----	102	1.000	----	----	102	1.000	----	----	102	0.912	0.088	----	----
Hamma Hamma River	36	0.833	0.167	----	----	36	1.000	----	----	----	36	0.569	0.347	0.056	0.028	----	----	36	1.000	----	36	1.000	----	----	36	1.000	----	----	36	1.000	----	----	36	0.931	0.069	----	----
Quilcene Bay & FH	239	0.935	0.075	----	----	240	0.979	0.021	----	----	240	0.600	0.267	0.110	0.023	----	----	240	0.996	0.004	240	1.000	----	----	240	1.000	----	----	240	1.000	----	----	239	0.874	0.126	----	----
Lilliwaup Creek	120	0.925	0.075	----	----	120	0.979	0.017	0.004	----	120	0.646	0.267	0.050	0.017	----	----	120	1.000	----	120	1.000	----	----	120	1.000	----	----	120	1.000	----	----	120	0.912	0.067	----	----
Northern Puget Sound																																					
Fall-run																																					
Skagit/Samish River																																					
Squire Creek	140	0.830	0.161	----	----	140	1.000	----	----	----	140	0.700	0.226	0.026	0.050	----	----	140	1.000	----	140	1.000	----	----	140	1.000	----	----	140	0.989	0.004	----	140	0.989	0.011	----	----
Jim Creek	148	0.903	0.097	----	----	151	0.997	0.003	----	----	148	0.611	0.307	0.027	0.051	----	0.003	151	0.997	0.003	151	1.000	----	----	151	1.000	----	----	149	0.997	----	0.003	151	0.987	0.013	----	----
Fortson Creek	211	0.841	0.159	----	----	211	0.995	0.005	----	----	209	0.608	0.282	0.041	0.067	----	0.014	211	1.000	----	211	1.000	----	----	211	0.996	----	0.002	211	0.991	0.009	----	211	0.981	0.019	----	----
N. F. Sollequemian River	140	0.986	0.132	----	----	139	1.000	----	----	----	139	0.666	0.209	0.060	0.065	----	0.011	140	1.000	----	140	1.000	----	----	140	0.996	0.004	----	140	1.000	----	----	139	0.978	0.022	----	----
Skagit River																																					
Skagit River	100	0.870	0.130	----	----	100	0.990	0.010	----	----	99	0.646	0.313	0.056	0.086	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	1.000	----	----	----
Baker Creek	99	0.883	0.117	----	----	99	1.000	----	----	----	99	0.633	0.255	0.030	0.081	----	0.015	99	1.000	----	99	1.000	----	----	99	1.000	----	----	99	0.995	0.005	----	99	0.990	0.010	----	----
Dan Creek	162	0.862	0.139	----	----	163	0.990	0.010	----	----	163	0.621	0.275	0.036	0.065	----	0.003	163	1.000	----	163	1.000	----	----	163	0.997	----	0.003	163	0.997	0.003	----	161	0.977	0.023	----	----
Finney Creek	41	0.805	0.085	----	----	41	1.000	----	----	----	41	0.646	0.220	0.049	0.085	----	----	41	1.000	----	41	1.000	----	----	41	1.000	----	----	41	1.000	----	----	41	0.963	0.037	----	----

Appendix (*Continued*)

Location	mDDH-1*					sDDH-1*					sDDH-3*							LDH-A1*			LDH-B1*				LDH-B2*			mMDH-3*				sMDH-A1*			
	(S)	100	80	140	20	(S)	100	70	130	112	(S)	100	36	65	25	110	28	(S)	-100	60	(S)	100	140	200	(S)	100	130	115	(S)	-100	-60	-120	(S)	100	200	10
Salmon River																																				
Salmon Fh	100	0.905	0.195	----	----	100	0.985	0.015	----	----	99	0.835	0.309	0.298	0.071	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	----
Thomas Creek	98	0.870	0.122	----	----	102	0.995	0.005	----	----	102	0.559	0.275	0.093	0.084	----	0.005	102	1.000	----	102	1.000	----	----	102	1.000	----	----	102	1.000	----	----	101	0.985	0.015	----	----
Bob Smith Creek	100	0.960	0.140	----	----	99	0.990	0.010	----	----	100	0.606	0.370	0.096	0.030	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.975	0.025	----	----
Nooteack River																																				
Nooteack River	96	0.833	0.167	----	----	96	1.000	----	----	----	95	0.643	0.243	0.071	0.114	----	0.029	96	1.000	----	96	1.000	----	----	96	1.000	----	----	96	1.000	----	----	96	1.000	----	----	----
Kendall Fh	200	0.827	0.172	----	----	200	0.995	0.005	----	----	199	0.809	0.239	0.065	0.093	----	0.005	200	1.000	----	200	0.995	----	0.005	200	1.000	----	----	199	0.997	0.003	----	200	1.000	----	----	----
Maple Creek	100	0.795	0.205	----	----	100	0.990	0.005	----	----	100	0.615	0.256	0.075	0.095	----	0.005	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	----
Bellingham Bay																																				
Chuckanut Creek	35	0.943	0.167	----	----	34	0.971	0.029	----	----	35	0.586	0.300	----	0.114	----	----	35	1.000	----	35	1.000	----	----	35	1.000	----	----	35	1.000	----	----	35	0.957	0.043	----	----
Skykomish River																																				
Schoolhouse Slough	90	0.900	0.094	----	----	96	0.984	0.016	----	----	96	0.672	0.193	0.068	0.062	----	0.005	96	1.000	----	96	1.000	----	----	96	1.000	----	----	96	1.000	----	----	96	0.995	0.005	----	----
Wallace River	144	0.833	0.167	----	----	144	0.986	0.010	0.003	----	144	0.625	0.240	0.073	0.062	----	----	144	0.997	0.003	144	1.000	----	----	144	0.997	0.003	----	143	1.000	----	----	144	0.979	0.021	----	----
Skykomish River	200	0.955	0.146	----	----	200	0.997	0.002	----	----	199	0.638	0.196	0.068	0.106	----	0.003	200	1.000	----	200	1.000	----	----	200	1.000	----	----	200	0.985	0.015	----	200	0.972	0.027	----	----
Strait of Juan de Fuca																																				
Fall-run																																				
Deep Creek	100	0.980	0.120	----	----	100	0.980	----	0.010	----	100	0.690	0.260	0.030	0.010	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	----
Lyre River	100	0.955	0.045	----	----	100	0.990	----	----	0.010	100	0.735	0.215	0.035	0.015	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.985	0.015	----	----
Pysht River	205	0.945	0.055	----	----	205	0.994	0.006	----	----	204	0.539	0.304	0.069	0.030	----	----	205	1.000	----	205	1.000	----	----	205	1.000	----	----	204	0.994	0.006	----	205	0.883	0.068	----	0.052
Sooke River	100	0.965	0.035	----	----	99	0.995	0.005	----	----	99	0.480	0.424	0.040	0.056	----	----	100	0.995	0.005	100	1.000	----	----	100	0.995	0.005	----	100	0.985	0.015	----	100	0.995	0.015	----	----
Summer-run																																				
Salmon Creek	50	0.980	0.120	----	----	50	1.000	----	----	----	50	0.460	0.360	0.010	0.120	----	----	50	1.000	----	50	1.000	----	----	50	1.000	----	----	50	1.000	----	----	50	0.950	0.050	----	----
Snow Creek	50	0.900	0.100	----	----	50	1.000	----	----	----	50	0.510	0.330	----	0.160	----	----	50	1.000	----	50	1.000	----	----	50	1.000	----	----	50	1.000	----	----	50	0.910	0.090	----	----
Jimmycomelaisy Creek	99	0.859	0.141	----	----	100	0.995	0.005	----	----	100	0.495	0.370	0.105	0.070	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.940	0.060	----	----
Coastal and Columbia River																																				
Fall-run																																				
Chehalis River																																				
Bessey River	100	0.960	0.040	----	----	100	0.995	0.005	----	----	100	0.500	0.345	0.025	0.030	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.980	0.010	----	----
Wynoochee River	22	0.955	0.045	----	----	22	1.000	----	----	----	22	0.545	0.319	0.045	0.091	----	----	22	0.955	0.045	22	1.000	----	----	22	1.000	----	----	22	1.000	----	----	22	1.000	----	----	----
Stevens Creek	100	0.945	0.055	----	----	100	0.995	0.005	----	----	95	0.537	0.284	0.116	0.063	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	150	0.990	0.010	----	----
Willapa Bay																																				
Ellsworth Creek	100	0.950	0.050	----	----	100	0.985	----	0.015	----	100	0.535	0.290	0.035	0.060	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.985	0.005	0.010	----
Bear Creek	100	0.960	0.020	----	----	100	0.995	----	0.005	----	99	0.586	0.369	0.015	0.040	----	----	100	0.990	0.010	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	0.010	----
Columbia River																																				
Hamilton Creek	100	0.955	0.045	----	----	100	0.980	0.020	----	----	100	0.740	0.185	0.060	0.015	----	----	100	1.000	----	100	0.990	0.010	----	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	----
Grays River	100	0.940	0.060	----	----	100	1.000	----	----	----	100	0.625	0.225	0.135	0.015	----	----	100	0.945	0.055	100	0.945	0.055	----	100	1.000	----	----	100	1.000	----	----	100	0.995	----	0.005	----
Coal Creek																																				
Coal Creek	98	0.937	0.062	----	----	99	0.995	----	0.005	----	95	0.595	0.469	0.016	0.011	----	----	99	0.990	0.020	99	1.000	----	----	99	1.000	----	----	99	1.000	----	----	99	1.000	----	----	----
Georgia Strait																																				
Fall-run																																				
Southern Vancouver Island																																				
Nanaimo River	100	0.925	0.075	----	----	100	0.995	0.005	----	----	100	0.535	0.350	0.085	0.080	----	----	100	0.995	0.005	100	1.000	----	----	100	0.995	0.005	----	100	0.975	0.025	----	100	0.995	0.005	----	----
Cowichan River	100	0.925	0.075	----	----	100	0.990	0.010	----	----	99	0.612	0.298	0.031	0.061	----	----	100	0.990	0.030	100	1.000	----	----	100	1.000	----	----	100	0.990	0.010	----	100	0.985	0.015	----	----
Chemainus River	100	0.955	0.045	----	----	100	1.000	----	----	----	99	0.540	0.319	0.040	0.101	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	1.000	----	----	----
Goldstream River	100	0.935	0.070	0.005	----	100	1.000	----	----	----	100	0.600	0.290	0.035	0.075	----	----	100	0.990	0.010	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.995	0.005	----	----
Central Vancouver Island																																				
Big Qualicum Fh	200	0.930	0.070	----	----	200	0.985	0.010	0.005	----	200	0.612	0.295	0.045	0.047	----	----	200	0.935	0.065	200	1.000	----	----	200	0.995	0.005	----	200	0.992	0.007	----	200	0.990	0.010	----	----
Little Qualicum	100	0.940	0.060	----	----	99	0.985	0.005	0.010	----	100	0.535	0.285	0.055	0.020	----	0.005	100	0.995	0.005	100	1.000	----	----	100	1.000	----	----	100	0.985	0.015	----	100	0.985	0.005	----	----
Puntledge Fh	100	0.945	0.055	----	----	100	0.995	0.005	----	----	99	0.657	0.247	0.030	0.065	----	----	100	0.995	0.005	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.990	0.010	----	----
Southern south coast																																				
Chesakmus River	50	0.900	0.040	----	----	100	1.000	----	----	----	100	0.540	0.340	0.080	0.040	----	----	100	0.995	0.005	100	1.000	----	----	100	1.000	----	----	100	0.995	0.010	----	100	0.990	0.010	----	----
Indian Arm	99	0.924	0.070	----	----	100	0.990	0.005	0.005	----	100	0.620	0.300	0.030	0.050	----	----	100	0.995	0.005	100	1.000	----	----	100	1.000	----	----	100	0.995	0.015	----	100	1.000	----	----	----
Central south coast																																				
Nemaum River	100	0.950	0.050	----	----	99	1.000	----	----	----	100	0.635	0.290	0.030	0.045	----	----	100	1.000	----	100	1.000	----	----	100	0.995	0.005	----	100	1.000	----	----	100	0.990	0.010	----	----
Tzoonie River	100	0.980	0.120	----	----	99	0.970	----	0.030	----	99	0.535	0.343	0.030	0.091	----	----	100	1.000	----	100	1.000	----	----	100	0.995	----	0.005	100	0.990	0.010	----	100	0.970	0.030	----	----
Stamman Fh	100	0.930	0.070	----	----	100	1.000	----	----	----	100	0.570	0.325	0.050	0.055	----	----	100	1.000	----	100	1.000	----	----	100	0.995	0.005	----	100	0.970	0.030	----	100	0.990	0.010	----	----
Fraser River																																				
Fall-run																																				
Lower																																				
Albouette River	100	0.925	0.075	----	----	99	0.990	0.010	----	----	99	0.667	0.207	0.061	0.066	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.990	0.010	----	----
Stave River	100	0.945	0.055	----	----	100	0.995	0.005	----	----	100	0.645	0.210	0.070	0.075	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.990	0.010	----	----
Central																																				
Chilliwack - Vedder Fh	100	0.895	0.110	----	0.005	100	0.990	0.010	----	----	100	0.630	0.260	0.055	0.055	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	1.000	----	----	----
Chehalis @ Harrison Fh	100	0.890	0.110	----	----	100	1.000	----	----	----	90	0.648	0.265	0.029	0.061	----	----	100	0.990	0.010	100	1.000	----	----	100	1.000	----	----	100	1.000	----	----	100	0.990	0.010	----	----
Inch Creek Fh	100	0.910	0.095	----	0.005	99	1.000	----	----	----	99	0.646	0.207	0.070	0.051	0.010	0.010	100	0.995	0.005	100	1.000	----	----	100	1.000	----	----	100	0.995	0.015	----	100	0.995	0.015	----	----
Weaver Creek	97	0.912	0.088	----	----	100	0.985	0.015	----	----	100	0.635	0.235	0.065	0.065	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.975	0.025	----	----
Chehalis Fh	100	0.930	0.070	----	----	100	0.995	0.005	----	----	97	0.525	0.290	0.035	0.062	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.995	0.005	----	----
Harrison River	97	0.943	0.057	----	----	100	0.985	0.015	----	----	97	0.567	0.304	0.072	0.056	----	----	100	1.000	----	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.985	0.015	----	----
Squakum Creek	100	0.935	0.075	----	----	100	1.000	----	----	----	100	0.570	0.305	0.060	0.065	----	----	100	0.980	0.020	100	1.000	----	----	100	1.000	----	----	100	0.980	0.020	----	100	0.980	0.020	----	----
Wahleach Creek	100	0.870	0.135	----	----	100	0.980	0.020	----	----	100	0.660	0.275	0.045	0.130	----	----	100	0.995	0.005	100	1.000	----	----	100	1.000	----	----	100	0.980	0.020	----	100	0.990	0.010	----	----
West Coast Vancouver Island																																				
Fall-run																																				
Nitinat River and Fh	370	0.959	0.041	----	----	379	0.989	0.001	----	----	370	0.410	0.312	0.089	0.159	0.009	0.003	370	1.000	----	379	1.000	----	----	370	1.000	----	----	373	0.989	0.020	----	370	0.976	0.025	----	----
Nahmint River	100	0.960	0.050	----	----	100	1.000	----	----	----	100	0.535	0.340	0.050	0.175	----	----	100	0.995	0.005	100	1.000	----	----	100	1.000	----	----	100	0.995	0.005	----	100	0.975	0.025	----	----
Sarita River	127	0.941	0.059	----	----	127	0.992	0.004	0.004	----	127	0.445	0.311	0.095	0.151	0.098	----	127	1.000	----	127	1.000	----	----	127	1.000	----	----	127	0.992	0.004	----	126	0.960	0.040	----	----

Appendix (*Continued*)

Location	sMDH-B1,2*						sAAT-2*			MR*				PEPA*			PEPB-1*						PGDH*			PGM-2*			sIDDO-1*			TF-1*			TF-3*		
	(R)	100	73	50	130	145	(R)	100	152	(R)	100	92	112	94	(R)	100	110	(R)	-100	-146	-126	-127	-73	-50	(R)	100	89	(R)	100	129	(R)	100	40	(R)	-100	-50	(R)	100	95	90	105
Southern Puget Sound																																								
Fall-run																																								
Southern																																								
Elwht Creek	250	0.897	0.003	----	----	----	250	0.709	0.283	250	0.624	0.176	----	----	102	1.000	----	240	0.713	0.141	0.106	0.036	0.002	----	250	0.994	0.006	250	1.000	----	248	1.000	----	250	1.000	----	250	0.996	0.002	----	----
Perry Creek	348	0.993	0.007	----	----	----	348	0.724	0.276	348	0.667	0.183	----	----	279	1.000	----	348	0.726	0.136	0.097	0.062	0.001	----	348	0.988	0.012	348	1.000	----	348	1.000	----	348	1.000	----	348	0.999	0.001	----	----
Skein Creek Fh	348	1.000	----	----	----	----	348	0.767	0.233	350	0.650	0.150	----	----	280	1.000	----	348	0.734	0.142	0.090	0.036	----	----	350	0.989	0.011	360	1.000	----	350	1.000	----	261	1.000	----	261	1.000	----	----	----
Carbon River	150	0.986	0.013	----	----	----	150	0.570	0.430	150	0.627	0.173	----	----	140	0.993	0.007	140	0.716	0.179	0.064	0.061	----	----	150	0.993	0.007	150	1.000	----	150	0.997	0.003	150	1.000	----	150	1.000	----	----	----
Fennel Creek	100	0.995	0.005	----	----	----	100	0.795	0.205	100	0.820	0.060	----	----	100	1.000	----	98	0.691	0.276	0.061	0.071	----	----	100	0.975	0.025	100	1.000	----	100	0.995	0.005	100	1.000	----	100	1.000	----	----	----
Skookum Creek	100	1.000	----	----	----	----	100	0.895	0.305	100	0.765	0.235	----	----	98	1.000	----	98	0.689	0.133	0.122	0.056	----	----	100	0.995	0.005	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	----	----
Little Creek	50	0.995	0.005	----	----	----	50	0.750	0.250	50	0.730	0.270	----	----	50	0.990	0.010	40	0.694	0.139	0.122	0.051	----	----	50	0.990	0.010	50	1.000	----	50	1.000	----	50	1.000	----	50	1.000	----	----	----
Rendorf Creek	50	1.000	----	----	----	----	50	0.780	0.220	50	0.830	0.170	----	----	50	1.000	----	47	0.734	0.128	0.117	0.021	----	----	50	0.990	0.010	50	1.000	----	50	1.000	----	50	1.000	----	50	1.000	----	----	----
Upper Skookum Creek	100	0.992	0.008	----	----	----	100	0.775	0.225	100	0.760	0.240	----	----	100	1.000	----	98	0.672	0.209	0.098	0.042	----	----	100	1.000	----	100	1.000	----	100	1.000	----	50	1.000	----	50	0.990	0.010	----	----
Sherwood, Rocky & Coulter Cr	198	0.995	0.005	----	----	----	198	0.741	0.259	198	0.782	0.208	----	----	0	----	----	196	0.703	0.168	0.086	0.062	----	----	198	0.991	0.009	198	1.000	----	198	1.000	----	198	1.000	----	198	1.000	----	----	----
Mill Creek	179	0.989	0.011	----	----	----	178	0.772	0.228	179	0.743	0.257	----	----	59	1.000	----	178	0.705	0.118	0.096	0.081	----	----	179	1.000	----	179	1.000	----	179	1.000	----	179	1.000	----	178	0.989	0.011	----	----
Kennedy Creek	200	0.995	0.005	----	----	----	200	0.760	0.240	200	0.760	0.240	----	----	101	1.000	----	200	0.717	0.142	0.100	0.040	----	----	200	0.947	0.052	200	1.000	----	200	1.000	----	200	1.000	----	200	1.000	----	----	----
Goldsborough Creek	200	0.995	0.005	----	----	----	197	0.731	0.269	200	0.775	0.225	----	----	200	1.000	----	200	0.707	0.157	0.052	0.082	----	----	200	0.977	0.022	200	1.000	----	200	1.000	----	200	1.000	----	200	1.000	----	----	----
Eldeep Peninsula																																								
Chico Creek	196	0.995	0.005	----	----	----	196	0.750	0.250	196	0.722	0.278	----	----	97	1.000	----	196	0.750	0.084	0.102	0.064	----	----	196	0.997	0.003	196	1.000	----	196	1.000	----	196	1.000	----	196	1.000	----	----	----
Gorst Creek	100	1.000	----	----	----	----	100	0.735	0.265	100	0.760	0.240	----	----	100	1.000	----	98	0.750	0.098	0.112	0.071	----	----	100	0.985	0.015	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	----	----
Summer-run																																								
Southern																																								
Johns Creek	100	1.000	----	----	----	----	99	0.791	0.209	100	0.905	0.095	----	----	100	1.000	----	100	0.695	0.190	0.070	0.065	----	----	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	100	0.995	0.005	----	----
Sherwood Creek	100	1.000	----	----	----	----	100	0.745	0.255	100	0.700	0.300	----	----	95	1.000	----	100	0.660	0.165	0.050	0.125	----	----	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	----	----
Coulter Creek Fh	100	0.995	0.005	----	----	----	100	0.630	0.170	100	0.740	0.260	----	----	0	----	----	100	0.715	0.095	0.125	0.075	----	----	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	100	0.990	0.010	----	----
Eldeep Peninsula																																								
Blackjack Creek	100	0.992	0.008	----	----	----	100	0.840	0.160	100	0.810	0.190	----	----	100	1.000	----	100	0.690	0.100	0.125	0.085	----	----	100	0.990	0.010	100	1.000	----	99	1.000	----	100	1.000	----	100	1.000	----	----	----
Winter-run																																								
Chambers Creek	100	0.997	0.003	----	----	----	100	0.805	0.195	100	0.780	0.220	----	----	0	----	----	100	0.690	0.065	0.015	0.030	----	----	100	0.985	0.015	100	1.000	----	100	1.000	----	99	1.000	----	99	1.000	----	----	----
Nisqually River	300	0.996	0.004	----	----	----	300	0.812	0.188	299	0.709	0.291	----	----	191	0.992	0.008	300	0.620	0.090	0.052	0.067	----	0.002	300	0.910	0.090	300	1.000	----	291	1.000	----	300	1.000	----	300	1.000	----	----	----
Hood Canal																																								
Fall-run																																								
Eastern																																								
Big Mission Creek	67	1.000	----	----	----	----	67	0.709	0.291	67	0.709	0.291	----	----	39	1.000	----	67	0.590	0.172	0.119	0.119	----	----	67	1.000	----	67	1.000	----	67	1.000	----	67	0.963	0.037	67	1.000	----	----	----
Little Mission Creek	51	1.000	----	----	----	----	51	0.814	0.186	51	0.863	0.137	----	----	2	1.000	----	51	0.647	0.118	0.069	0.167	----	----	51	0.990	0.010	51	1.000	----	51	1.000	----	51	0.980	0.020	51	1.000	----	----	----
Big Beef Creek	100	0.997	0.003	----	----	----	100	0.765	0.235	100	0.870	0.130	----	----	100	0.995	0.005	89	0.611	0.217	0.061	0.121	----	----	100	0.985	0.015	100	1.000	----	100	1.000	----	100	0.995	0.005	100	1.000	----	----	----
Dewatto River	251	0.990	0.010	----	----	----	250	0.794	0.206	252	0.851	0.149	----	----	224	1.000	----	249	0.610	0.219	0.084	0.086	----	----	253	0.990	0.010	251	1.000	----	242	0.996	0.004	252	0.984	0.008	252	1.000	----	----	----
Tahuya River	90	0.987	0.012	----	----	----	90	0.706	0.294	90	0.856	0.144	----	----	90	1.000	----	90	0.697	0.106	0.091	0.119	0.006	----	90	0.994	0.006	90	1.000	----	90	0.994	0.006	90	0.991	0.019	90	1.000	----	----	----
Western																																								
Eneas Fh	199	0.997	0.503	----	----	----	199	0.799	0.229	199	0.860	0.110	----	----	0	1.000	----	199	0.668	0.146	0.106	0.095	----	----	199	0.995	0.005	199	1.000	----	199	1.000	----	199	0.995	----	199	0.995	----	0.005	----
Hood Canal Fh	449	0.994	0.506	----	----	----	443	0.777	0.223	449	0.872	0.128	----	----	196	1.000	----	449	0.569	0.227	0.099	0.104	0.002	----	449	0.978	0.022	449	1.000	----	449	0.998	0.001	449	1.000	----	449	1.000	----	----	----
Minter Creek Fh	100	0.990	0.010	----	----	----	99	0.808	0.192	100	0.845	0.155	----	----	0	----	----	100	0.595	0.215	0.095	0.105	----	----	100	0.990	0.010	100	1.000	----	100	1.000	----	100	0.990	0.010	100	1.000	----	----	----
Lilliwaup Creek	94	0.992	0.008	----	----	----	82	0.799	0.201	94	0.862	0.138	----	----	0	----	----	94	0.670	0.207	0.085	0.074	----	----	94	0.995	0.005	94	1.000	----	94	1.000	----	94	1.000	----	94	1.000	----	----	----
Lower Elwha Fh	100	1.000	----	----	----	----	100	0.800	0.200	100	0.870	0.130	----	----	0	----	----	100	0.750	0.145	0.065	0.040	----	----	100	0.995	0.010	100	1.000	----	100	0.990	0.010	100	1.000	----	100	1.000	----	----	----
Tulalip Fh	197	0.996	0.004	----	----	----	196	0.793	0.217	197	0.850	0.150	----	----	98	1.000	----	197	0.699	0.157	0.109	0.046	----	----	197	0.995	0.005	197	1.000	----	197	0.997	0.003	197	0.997	0.003	197	1.000	----	----	----
Wallace Fh	100	1.000	----	----	----	----	100	0.805	0.195	100	0.865	0.135	----	----	100	1.000	----	99	0.712	0.136	0.086	0.066	----	----	100	0.995	0.005	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	----	----
Kelsa Creek Fh	150	0.981	0.099	----	----	----	149	0.772	0.228	148	0.809	0.191	----	----	51	1.000	----	150	0.537	0.223	0.117	0.123	----	----	150	0.997	0.003	150	1.000	----	150	1.000	----	150	1.000	----	150	1.000	----	----	----
McKernan Fh	100	0.995	0.005	----	----	----	99	0.791	0.209	100	0.845	0.155	----	----	0	----	----	100	0.625	0.190	0.095	0.100	----	----	100	0.990	0.020	100	1.000	----	100	0.995	0.005	100	0.995	0.005	100	1.000	----	----	----
Big Quilcene Fh	150	0.997	0.999	----	----	----	98	0.730	0.270	100	0.875	0.125	----	----	59	1.000	----	100	0.590	0.200	0.170	0.035	0.005	----	100	0.995	0.005	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	----	----
Bellingham Mainline Fh	100	0.990	0.010	----	----	----	99	0.758	0.242	100	0.910	0.090	----	----	99	0.995	0.005	100	0.730	0.145	0.075	0.050	----	----	100	0.995	0.005	100	1.000	----	100	0.970	0.030	99	1.000	----	99	1.000	----	----	----
Hamma Hamma River	196	0.993	0.007	----	----	----	196	0.769	0.231	194	0.826	0.174	----	----	196	1.000	----	196	0.632	0.162	0.092	0.114	----	----	196	0.989	0.011	196	1.000	----	196	0.993	0.011	196	0.997	0.003	196	1.000	----	----	----
Dosewallips River	105	0.993	0.007	----	----	----	105	0.733	0.267	105	0.843	0.157	----	----	105	1.000	----	103	0.675	0.141	0.092	0.092	----	----	105	0.995	0.005	105	1.000	----	105	0.995	0.005	105	0.995	0.005	105	1.000	----	----	----
Skokomish wild																																								
Vance Creek	101	0.995	0.005	----	----	----	101	0.728	0.272	101	0.822	0.178	----	----	51	1.000	----	101	0.629	0.193	0.079	0.099	----	----	101	0.995	0.005	101	1.000	----	101	1.000	----	101	1.000	----	101	1.000	----	----	----
NF Skokomish River	111	0.990	0.011	----	----	----	111	0.793	0.207	111	0.863	0.117	----	----	111	1.000	----	111	0.669	0.169	0.090	0.163	----	----	111	0.991	0.009	111	1.000	----	111	0.991	0.009	111	0.991	0.009	111	1.000	----	----	----
Summer-run																																								
Eastern																																								
Union River	100	0.983	0.016	----	----	----	100	0.805	0.195	100	0.675	0.325	----	----	0	----	----	100	0.715	0.030	0.130	0.085	----	----	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	100	1.000	----	----	----
Western																																								
Duckabush River	125	0.874	0.026	----	----	----	125	0.884	0.116	126	0.864	0.336	----	----	95	1.000	----	126	0.690	0.084	0.068	0.168	----	----	125	1.000	----	125	1.000	----	125	1.000	----	123	1.000	----	123	1.000	----	----	----
Dosewallips River	102	0.983	0.017	----	----	----	102	0.853	0.147	102	0.701	0.299	----	----	102	1.000	----	81	0.632	0.011	0.240	0.016	----	----	102	1.000	----	102	1.000	----	102	1.000	----	102	1.000	----	102	1.000	----	----	----
Hamma Hamma River	36	0.965	0.034	----	----	----	36	0.861	0.139	36	0.811	0.389	----	----	13	1.000	----	36	0.653	0.083	0.093	0.191	----	----	36	1.000	----	36	1.000	----	36	1.000	----	36	1.000	----	36	1.000	----	----	----
Quilcene Bay & Fh	240	0.960	0.039	----	----	----	240	0.825	0.075	240	0.627	0.373	----	----	240	1.000	----	239	0.676	0.063	0.138	0.136	----	----	240	1.000	----	240	1.000	----	240	1.000	----	239	1.000	----	239	1.000	----	----	----
Lilliwaup Creek	120	0.977	0.023	----	----	----	120	0.929	0.071	120	0.596	0.404	----	----	72	1.000	----	119	0.813	0.071	0.239	0.176	----	----	120	1.000	----	120	1.000	----	120	1.000	----	120	1.000	----	120	1.000	----	----	----
Northern Puget Sound																																								
Fall-run																																								
Skilaguamish River																																								
Squire Creek	140	0.996	0.002	----	----	----	139	0.739	0.261	140	0.864	0.196	----	----	121	1.000	----	139	0.705	0.140	0.122	0.032	----	----	140	0.964	0.029	140	0.986	0.014	81	0.988	0.012	140	1.000	----	126	1.000	----	----	----
Jim Creek	149	0.996	0.002	----	----	----	151	0.802	0.306	151	0.776	0.223	----	----	111	1.000	----	150	0.777	0.147	0.067	0.020	----	----	151	0.993	0.007	151	1.000	----	151	0.997	0.003	151	1.000	----	151	1.000	----	----	----
Portsen Creek	211	0.990	0.010	----	----	----	210	0.688	0.312	211	0.813	0.187	----	----	179	0.994	0.006	210	0.752	0.121	0.063	0.033	----	----	211	0.972	0.028	211	0.986	0.006	211	1.000	----	211	1.000	----	211	1.000	----	----	----
N. F. Skilaguamish River	140	1.000	----	----	----	----	140	0.696	0.304	140	0.807	0.193	----	----	136	0.996	0.004	139	0.712	0.144	0.097	0.047	----	----	140	0.993	0.007	140	0.993	0.007	140	0.993	0.007	140	1.000	----	140	1.000	----	----	----
Skagit River																																								
Skagit River	100	0.992	0.008	----	----	----	100	0.725	0.275	100	0.830	0.170	0.005	----	99	0.990	0.010	100	0.685	0.165	0.130	0.040	----	----	100	0.975	0.025	99	0.995	0.005	100	1.000	----	100	1.000	----	100	1.000	----	----	----
Baker Creek	99	0.995	0.005	----	----	----	99	0.689	0.311	99	0.857	0.143	----	----	99	1.000	----	99	0.788	0.126	0.071	0.041	----	----	99	0.990	0.015	99	0.990	0.010	99	1.000	----	99	1.000	----	99	1.000	----	----	----
Dan Creek	153	1.000	----	----	----	----	153	0.748	0.252	153	0.797	0.203	----	----	153	0.987	0.013	151	0.805	0.093	0.079	0.023	----	----	153	0.987	0.013	153	0.993	0.007	153	1.000	----	151	1.000	----	151	1.000	----	----	----
Finney Creek	41	0.994	0.006	----	----	----	41	0.805	0.195	41	0.951	0.049	----	----	41	1.000	----	39	0.603	0.291	0.164	0.013	----	----	41	1.000	----	41	1.000	----	41	1.000	----	41	1.000	----	41	1.000	----	----	----

Appendix (*Concluded*)

Location	*sMDH-B1,2** (N)	100	72	60	120	140	*sMEP-3** (N)	100	122	*MPI** (N)	100	82	113	84	*PEPA** (N)	100	110	*PEPB-1** (N)	-100	-140	-136	-127	-72	-60	*PGDH** (N)	100	90	*PGM-2** (N)	100	120	*sODD-1** (N)	100	40	*TPI-1** (N)	-100	-80	*TPI-2** (N)	100	85	80	106
Semiahmoo River																																								
Semiahmoo FH	100	0.930	0.010	—	—	—	100	0.910	0.150	100	0.965	0.135	—	—	100	1.000	—	100	0.975	0.210	0.050	0.065	—	—	100	0.995	0.005	100	1.000	—	100	1.000	—	99	1.000	—	99	1.000	—	—	—
Thomas Creek	102	0.995	0.005	—	—	—	99	0.899	0.301	102	0.992	0.100	—	—	100	0.995	0.005	101	0.757	0.119	0.059	0.064	—	—	102	0.995	0.005	99	1.000	—	101	0.990	0.010	102	1.000	—	102	1.000	—	—	—
Bob Smith Creek	100	0.992	0.005	—	—	0.003	100	0.775	0.225	100	0.900	0.100	—	—	100	1.000	—	99	0.991	0.210	0.073	0.047	—	—	100	1.000	—	100	1.000	—	100	0.995	0.005	99	1.000	—	99	1.000	—	—	—
Nooksack River																																								
Nooksack River	36	1.000	—	—	—	—	36	0.788	0.214	36	0.875	0.125	—	—	36	1.000	—	36	0.953	0.208	0.056	0.083	—	—	36	1.000	—	36	1.000	—	36	1.000	—	34	1.000	—	34	1.000	—	—	—
Kendall FH	200	1.000	—	—	—	—	200	0.795	0.205	200	0.895	0.105	—	—	184	0.989	0.011	200	0.735	0.140	0.057	0.065	—	0.002	200	0.992	0.007	200	1.000	—	200	1.000	—	200	1.000	—	200	0.997	0.002	—	—
Maple Creek	100	1.000	—	—	—	—	100	0.760	0.240	100	0.945	0.155	—	—	0	—	—	99	0.758	0.068	0.106	0.071	—	—	100	0.975	0.035	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—
Bellingham Bay																																								
Chuckanut Creek	36	1.000	—	—	—	—	36	0.729	0.271	36	0.900	0.200	—	—	36	1.000	—	34	0.574	0.265	0.118	0.044	—	—	36	1.000	—	36	1.000	—	36	1.000	—	36	1.000	—	36	1.000	—	—	—
Skagit River																																								
Schoolhouse Slough	98	0.995	0.005	—	—	—	98	0.750	0.250	98	0.991	0.100	—	—	96	0.995	0.005	98	0.706	0.109	0.130	0.052	—	—	99	1.000	—	96	0.990	0.010	98	0.995	0.005	99	1.000	—	96	1.000	—	—	—
Wallace River	144	0.995	0.005	—	—	—	144	0.733	0.267	144	0.795	0.201	—	—	144	0.997	0.003	142	0.792	0.113	0.048	0.049	—	—	144	0.995	0.014	144	0.986	0.014	144	1.000	—	144	1.000	—	144	1.000	—	—	—
Skagit River	200	0.991	0.009	—	—	—	200	0.717	0.282	199	0.914	0.166	—	—	200	1.000	—	199	0.693	0.153	0.113	0.040	—	—	200	0.987	0.012	200	0.985	0.015	200	0.997	0.002	200	1.000	—	200	1.000	—	—	—
Strait of Juan de Fuca																																								
Fall-run																																								
Deep Creek	100	1.000	—	—	—	—	100	0.880	0.320	100	0.960	0.135	—	0.005	80	1.000	—	98	0.033	0.179	0.102	0.067	—	—	100	1.000	—	100	1.000	—	100	1.000	—	100	0.960	0.005	—	0.016			
Lyre River	100	1.000	—	—	—	—	100	0.745	0.255	100	0.965	0.135	—	—	80	1.000	—	100	0.005	0.210	0.100	0.050	0.025	—	100	1.000	—	100	1.000	—	100	1.000	—	100	0.985	—	—	0.016			
Pysht River	265	0.930	0.002	—	—	—	265	0.775	0.225	265	0.909	0.052	—	—	211	1.000	—	264	0.003	0.191	0.095	0.075	0.008	—	265	1.000	—	265	1.000	—	265	1.000	—	264	1.000	—	—	—			
Sooke River	100	0.995	0.005	—	—	—	100	0.810	0.190	100	0.955	0.095	—	—	0	—	—	100	0.025	0.175	0.130	0.070	—	—	100	1.000	—	100	1.000	—	100	1.000	—	0	—	—	0	—	—	—	—
Summer-run																																								
Salmon Creek	50	0.960	0.020	—	—	—	50	0.960	0.040	50	0.810	0.300	—	—	50	1.000	—	50	0.020	0.120	0.020	0.040	—	—	50	1.000	—	50	1.000	—	50	1.000	—	50	1.000	—	50	1.000	—	—	—
Snow Creek	50	1.000	—	—	—	—	50	0.950	0.050	50	0.920	0.360	—	—	50	1.000	—	50	0.730	0.130	0.110	0.030	—	—	50	1.000	—	50	1.000	—	50	1.000	—	49	1.000	—	—	—	—	—	—
Jimmycomelately Creek	100	0.992	0.008	—	—	—	100	0.925	0.075	100	0.715	0.265	—	—	3	1.000	—	100	0.990	0.110	0.140	0.065	—	—	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—	—	—	—
Cassiar and Columbia River																																								
Fall-run																																								
Chehalis River																																								
Bessop River	100	0.957	0.042	—	—	—	100	0.690	0.310	100	0.926	0.070	—	0.005	32	1.000	—	100	0.990	0.225	0.020	0.090	—	0.005	97	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—			
Wynoochee River	22	0.977	0.022	—	—	—	22	0.614	0.386	22	0.964	0.136	—	—	22	1.000	—	22	0.932	0.227	0.045	0.045	—	—	22	1.000	—	22	1.000	—	22	1.000	—	22	1.000	—	—	—			
Stevens Creek	100	0.975	0.025	—	—	—	100	0.650	0.350	100	0.920	0.075	0.005	—	70	1.000	—	100	0.995	0.320	0.035	0.090	—	—	100	0.995	0.005	100	1.000	—	100	1.000	—	100	1.000	—	—	—			
Willapa Bay																																								
Ellsworth Creek	100	0.952	0.099	—	—	—	100	0.690	0.310	100	0.890	0.110	—	—	13	1.000	—	100	0.585	0.230	0.090	0.120	0.005	—	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—			
Bear Creek	100	0.965	0.035	—	—	—	100	0.675	0.325	100	0.900	0.100	—	—	20	1.000	—	100	0.610	0.265	0.040	0.095	—	—	99	1.000	—	100	1.000	—	100	1.000	—	100	0.895	—	—	0.005			
Columbia River																																								
Hamilton Creek	100	0.990	0.010	—	—	—	99	0.596	0.404	99	0.948	0.152	—	—	99	1.000	—	98	0.000	0.224	0.041	0.040	—	—	100	0.995	0.005	100	1.000	—	100	1.000	—	100	1.000	—	—	—			
Grays River	100	0.990	0.010	—	—	—	100	0.695	0.315	100	0.995	0.105	—	—	100	1.000	—	96	0.703	0.193	0.031	0.073	—	—	100	1.000	—	100	1.000	—	99	1.000	—	99	1.000	—	—	—			
Coal Creek																																								
Coal Creek	99	0.947	0.053	—	—	—	99	0.622	0.378	99	0.940	0.051	—	—	99	1.000	—	98	0.000	0.273	0.058	0.110	—	—	99	1.000	—	99	1.000	—	99	1.000	—	99	1.000	—	—	—			
Georgia Strait																																								
Fall-run																																								
Southern Vancouver Island																																								
Nanaimo River	100	1.000	—	—	—	—	100	0.705	0.215	100	0.920	0.080	—	—	0	—	—	100	0.046	0.175	0.145	0.035	—	—	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—
Cowichan River	100	0.997	—	—	0.003	—	100	0.780	0.220	100	0.900	0.095	—	0.005	0	—	—	100	0.015	0.205	0.195	0.025	—	—	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—
Chemainus River	100	1.000	—	—	—	—	100	0.835	0.165	100	0.870	0.130	—	—	0	—	—	100	0.050	0.240	0.085	0.010	—	0.005	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—
Goldstream River	100	0.997	—	—	0.003	—	100	0.715	0.285	100	0.890	0.120	—	—	100	1.000	—	99	0.596	0.167	0.167	0.045	0.005	—	100	1.000	—	100	1.000	—	100	0.990	0.020	99	1.000	—	99	1.000	—	—	—
Central Vancouver Island																																								
Big Qualicum FH	200	0.995	0.005	—	—	—	200	0.797	0.202	200	0.940	0.060	—	—	152	1.000	—	200	0.502	0.260	0.130	0.025	—	0.002	199	0.997	0.003	200	0.997	—	200	0.995	0.005	200	1.000	—	200	1.000	—	—	—
Little Qualicum	100	0.997	0.003	—	—	—	100	0.775	0.225	100	0.900	0.100	—	—	100	1.000	—	99	0.636	0.167	0.177	0.030	—	—	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—
Puntledge FH	100	0.997	0.003	—	—	—	100	0.765	0.235	100	0.880	0.120	—	—	100	1.000	—	100	0.520	0.220	0.145	0.010	0.005	—	100	0.995	0.005	100	1.000	—	98	1.000	—	98	1.000	—	—	—			
Southern south coast																																								
Cheakamus River	100	0.997	0.003	—	—	—	100	0.750	0.250	100	0.975	0.125	—	—	0	—	—	99	0.778	0.146	0.081	0.015	—	—	100	1.000	—	100	1.000	—	100	0.995	0.005	100	1.000	—	—	—			
Indian Arm	100	0.992	0.008	—	—	—	100	0.760	0.240	100	0.925	0.075	—	—	0	—	—	97	0.727	0.160	0.077	0.015	—	—	100	0.995	0.005	100	1.000	—	100	1.000	—	100	0.990	0.010	100	1.000	—	—	—
Central south coast																																								
Mamquam River	100	0.995	0.005	—	—	—	100	0.765	0.235	99	0.939	0.081	—	—	100	1.000	—	100	0.705	0.210	0.075	0.010	—	—	100	1.000	—	100	1.000	—	99	1.000	—	99	1.000	—	—	—			
Toquina River	100	0.997	—	—	—	0.003	100	0.780	0.220	100	0.890	0.110	—	—	100	1.000	—	100	0.700	0.195	0.095	0.020	—	—	100	1.000	—	100	1.000	—	99	1.000	—	99	1.000	—	—	—			
Shammon FH	100	1.000	—	—	—	—	100	0.795	0.205	100	0.895	0.100	—	0.005	99	1.000	—	100	0.635	0.160	0.105	0.035	—	0.005	100	0.980	0.015	100	1.000	—	100	0.995	0.005	100	1.000	—	100	1.000	—	—	—
Fraser River																																								
Fall-run																																								
Lower																																								
Alouette River	100	0.995	0.003	0.003	—	—	100	0.860	0.140	100	0.885	0.115	—	—	0	—	—	100	0.000	0.200	0.105	0.035	—	—	100	0.995	0.005	100	1.000	—	100	1.000	—	100	1.000	—	100	0.990	—	0.010	—
Stave River	100	0.985	0.012	0.003	—	—	100	0.835	0.165	100	0.895	0.105	—	—	0	—	—	99	0.002	0.250	0.122	0.020	—	—	100	0.990	0.010	100	1.000	—	100	1.000	—	100	0.990	—	100	0.990	—	0.010	—
Central																																								
Chilliwack - Vedder FH	100	0.995	0.015	—	—	—	100	0.815	0.185	100	0.990	0.100	—	—	100	1.000	—	100	0.685	0.195	0.100	0.050	—	—	100	0.990	0.005	100	1.000	—	100	1.000	—	100	1.000	—	—	—			
Chehalis @ Harrison FH	100	0.992	0.008	—	—	—	100	0.840	0.160	100	0.945	0.155	—	—	31	0.984	0.016	100	0.040	0.160	0.135	0.055	—	—	100	1.000	—	100	1.000	—	70	0.993	0.007	100	1.000	—	70	1.000	—	—	—
Inch Creek FH	100	0.997	0.003	—	—	—	100	0.865	0.135	100	0.910	0.090	—	—	59	1.000	—	100	0.715	0.195	0.080	0.055	—	0.005	100	0.995	0.005	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—
Weaver River	100	0.983	0.016	—	—	—	100	0.850	0.150	100	0.870	0.130	—	—	0	—	—	100	0.040	0.165	0.130	0.055	—	—	100	0.995	0.005	100	1.000	—	100	1.000	—	0	—	—	0	—	—	—	—
Chehalis FH	100	0.992	0.008	—	—	—	100	0.870	0.130	100	0.920	0.080	—	—	100	1.000	—	98	0.000	0.140	0.122	0.041	—	—	100	0.990	0.010	100	1.000	—	100	0.995	0.005	100	1.000	—	100	0.995	—	0.005	—
Harrison River	98	0.997	0.003	—	—	—	100	0.905	0.095	100	0.885	0.115	—	—	100	0.995	0.005	100	0.715	0.145	0.085	0.055	—	—	100	0.995	0.005	100	1.000	—	100	0.995	0.005	100	1.000	—	100	1.000	—	—	—
Squamish River	100	0.992	0.008	—	—	—	100	0.900	0.200	100	0.945	0.105	—	—	100	0.995	0.015	97	0.675	0.113	0.134	0.077	—	—	100	0.995	0.015	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—
Wahleach Creek	100	0.997	0.003	—	—	—	100	0.830	0.170	100	0.890	0.110	—	—	0	—	—	100	0.095	0.160	0.105	0.060	—	—	100	0.995	0.005	100	1.000	—	100	1.000	—	100	1.000	—	100	1.000	—	—	—
West Coast Vancouver Island																																								
Fall-run																																								
Nitinat River and FH	379	0.995	0.005	—	—	—	379	0.770	0.230	378	0.919	0.091	—	—	102	1.000	—	376	0.501	0.369	0.129	0.019	—	—	378	0.996	0.003	369	1.000	—	369	1.000	—	370	0.989	0.001	370	0.999	0.004	—	—
Harrison River	100	0.997	0.012	—	—	—	100	0.840	0.160	100	0.920	0.080	—	—	100	1.000	—	100	0.695	0.270	0.110	0.010	—	0.005	100	0.995	0.005	100	1.000	—	100	1.000	—	0	—	—	0	—	—	—	—
Somass River	127	0.988	0.010	0.002	—	—	127	0.760	0.317	126	0.944	0.056	—	—	127	1.000	—	123	0.577	0.317	0.089	0.060	—	—	127	0.996	0.004	121	1.000	—	127	1.000	—	100	1.000	—	99	1.000	—	—	—

*To condense this appendix, the number of heterozygotes (in parentheses) at each location for the following loci are footnoted: *CK-A1*70* (1) in Union River; *CK-A1*144* (2) in Ellsworth Creek; *GPI-A*95* (1) in Harrison River; *G3PDH-1*-76* (5) in Pysht River, (1) in Sooke River, (6) in Nitinat River and FH; *G3PDH-1*-110* (2) in Goldstream River; *LDH-A2*65* (2) Ellsworth Creek, (1) Grays River; *sMDH-A2-15* (2) in Elson Creek FH, (1) Hood Canal FH, (1) in Lilliwaup Creek; *PGDH*104* (1) Lower Elwha FH, (1) Squire Creek, (1) Sliammon FH; *PGDH*93* (1) Squire Creek, (1) Chilliwack-Vedder FH, (1) Nitinat River and FH; *PGDH*110* (1) Illabot Creek; *PGM-2*75* (1) Big Qualicum FH; *TPI-2*85* (2) Cheakamus River; *TPI-4*104* (1) Johns Creek, (6) Nisqually River, (2) Pysht River; *TPI-4*91* (1) Wahleach Creek.

Genetic Stock Structure of Western Alaska Chum Salmon and a Comparison with Russian Far East Stocks

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Substantial genetic divergence was found among chum salmon (*Oncorhynchus keta*) populations collected from North America and Russia. Five major groups of populations can be identified by geographic region: (1) lower Yukon River summer run; (2) upper Yukon River fall run; (3) Bristol Bay area; (4) Alaska Peninsula; and (5) Russia. Mean heterozygosities were 0.064, 0.062, 0.065, 0.064, and 0.063, respectively; and the percent polymorphic loci values at the 0.99 level were 33.7, 31.3, 32.6, 30.6, and 30.9%, respectively. The hierarchical gene diversity analysis showed that 95.42% of the diversity can be explained by heterogeneity within sites, 1.36% among sites, 0.49% between Yukon River run timing, 1.69% among areas, and 1.04% among countries. The Alaska Peninsula populations are genetically more similar to populations from Russia than to those from western Alaska, and two populations from the upper Yukon River are distinct from other nearby populations. Possible reasons for these findings concern the complex glacial histories of the watersheds.

Les auteurs ont décelé une importante divergence génétique entre des populations de saumon kéta (*Oncorhyrchus keta*) de l'Amérique du Nord et de la Russie. Ils ont identifié cinq grandes populations d'après la région géographique : (1) la remonte d'été dans le cours inférieur du fleuve Yukon, (2) la remonte d'automne dans le cours supérieur du fleuve Yukon, (3) la région de la baie de Bristol, (4) la péninsule de l'Alaska, et (5) la Russie, populations pour lesquelles ils ont déterminé une hétérozygosité moyenne respective de 0,064, 0,062, 0,065, 0,064 et 0,063, et un pourcentage respectif de loci polymorphes à un niveau de confiance de 0,99 de 33,7, 31,3, 32,6, 30,6 et 30,9 %. L'analyse hiérarchique de la diversité génétique a révélé que l'hétérogénéité intra-site en expliquait 95,42 %; l'hétérogénéité inter-sites, 1,36 %; l'hétérogénéité entre le moment de la montaison dans le Yukon, 0,49 %; l'hétérogénéité inter-régions, 1,69 %; et l'hétérogénéité inter-pays, 1,04 %. Au plan génétique, les populations de la péninsule de l'Alaska ressemblent plus aux populations de la Russie qu'à celles de l'ouest de l'Alaska, tandis que deux populations du cours supérieur du Yukon sont différentes de populations voisines. L'histoire complexe de la glaciation dans ces bassins versants est peut-être à l'origine de ces différences.

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Chum salmon (*Oncorhynchus keta*) have the widest natural distribution of all the salmon species (Bakkala 1970; Fredin et al. 1977). In Asia they are found from the Naktong River in Korea, north through Japan and Russia, to the Lena River on the Arctic coast (Salo 1991). In North America they range from the San Lorenzo River in California north through Oregon, Washington, British Columbia, Alaska, and the Arctic Ocean as far as the McKenzie River in the Northwest Territories (Dymond 1940; Wynne-Edwards 1952).

Chum salmon migrate to the ocean immediately after emergence from the gravel and 2–5 yr later return to their natal rivers to spawn. Early and late, or summer-run and fall-run, races exist throughout the entire species range (Salo 1991). Two river systems, the Amur in Asia and the Yukon in North America, support both summer-run and fall-run populations. In the Yukon River, summer-run fish enter the river from early June to mid-July and spawn throughout the summer months in the lower and mid-river tributaries. Fall-run fish enter the river from mid-July to early September and spawn in September and October in the middle and upper tributaries of the Yukon River (Buklis 1981). The chum salmon runs of the remainder of western Alaska are considered summer-run fish, as are the runs in the Anadyr, Kanchalan, and Magadan rivers in Russia. Chum salmon are not usually known for long-distance freshwater migrations. However, the Amur River and the Yukon River support spawning populations that migrate great distances upriver to spawn. Fall-run chum salmon migrate nearly 3000 km up the Yukon River to spawning areas in the Yukon Territory of Canada (Beacham et al. 1988).

Ocean distribution as shown by tag recoveries indicates that Asian chum salmon migrate much greater distances than North American chum salmon. Asian chum salmon have been recovered as far east as 140°E in the Gulf of Alaska while North American chum salmon have not been recovered much further west than 175°W (Salo 1991). There is, therefore, a large area of overlap in the oceanic distribution of stocks from Asia and North America.

A great deal of interest has been shown in methods to discriminate among stocks of chum salmon for several reasons. The high-seas squid fishery harvests large numbers of chum salmon of unknown origin (Helle 1989). Researchers in the United States and Canada suspect that a large proportion of these fish are of North American origin. Allocation disputes also create problems between the United States and Canada in the Yukon River, at border areas between southeast Alaska and northern British Columbia, and between southern British Columbia and the state of Washington. Competition between various user groups within countries is also of major concern.

Two methods have been used to discriminate among stocks of chum salmon and to estimate the stock composition of mixed-stock fisheries: scale pattern analysis (SPA), and genetic stock identification (GSI). SPA has been used with limited success in separating groups of chum salmon by continent of origin (Meyers et al. 1993). However, SPA requires yearly updates of each stock's scale patterns, and the short freshwater residence time of chum salmon may preclude discriminating between stocks. GSI is a method that relies on stock-specific differences in the relative frequencies of occurrence of genes (alleles) coding proteins detected by protein electrophoresis. It has been used on the west coast of North America to provide direct estimates of stock contributions to mixed-stock salmon fisheries (Grant et al. 1980; Okazaki 1981; Wishard 1981; Miller et al. 1983; Beacham et al. 1985; Shaklee et al. 1990b). In 1987, the U.S. Fish and Wildlife Service (FWS) became involved in describing the genetic stock structure of chum salmon stocks in western Alaska, and to evaluate the potential for using GSI in the management of Yukon River chum and chinook salmon (*O. tshawytscha*) (Wilmot et al. 1992). The FWS also became involved in cooperative work with the National Marine Fisheries Service and the Pacific Research Institute of Fisheries and Oceanography in Petropavlovsk–Kamchatsky (KoTINRO) and in Magadan (MoTINRO) in Russia to develop a genetic baseline for salmon species in the North Pacific Ocean. Here we describe our work on the genetic stock structure of western Alaska chum salmon and compare this relationship to three stocks of chum salmon from the Russian Far East.

Methods

Populations of chum salmon were sampled from Yukon River tributaries by the FWS, Alaska Department of Fish and Game, and the Canadian Department of Fisheries and Oceans. Samples from the Bristol Bay area and the Alaska Peninsula were collected by FWS personnel. The three collections from Russia were taken by personnel from the KoTINRO, and MoTINRO laboratories. Collections were made on the spawning grounds preferentially, and from main-stem weir or sonar sites when logistically necessary (Fig. 1, Table 1). We attempted to obtain 100 adults whenever possible from each drainage.

Four tissues (muscle, liver, heart, and eye) were taken from each fish for protein electrophoresis. Tissue samples were placed in labeled tubes, frozen on dry ice or liquid nitrogen, shipped to the FWS laboratory in Anchorage, Alaska, and stored at −70°C. The collections from Russia were put on ice and shipped to the KoTINRO laboratory in Petropavlovsk and held in liquid nitrogen until they could be brought to Anchorage. Protein electrophoresis followed methods described by Aebersold et al. (1987) and gene nomenclature followed recommendations of Shaklee et al. (1990a). A total of 48 proteins coded by 70 genetic loci were screened in chum salmon (Table 2). Twenty-four loci were used in the analyses. The loci were selected based on four criteria: (1) they must have been analyzed and scored for all populations; (2) the allelic variability must have been observed in at least one collection; (3) the observed banding pattern must have conformed to known models of inheritance among salmonids; and (4) the patterns must have been repeatable among tissues.

For each collection, the observed genotypic distribution at each locus was tested against the proportions expected in a random-mating, randomly sampled population (Hardy–Weinberg equilibrium) with a χ^2 goodness-of-fit test. Loci not in equilibrium were retested using bootstrap resampling to generate an empirical distribution ($n = 2000$) for the χ^2 under the null hypothesis of Hardy–Weinberg equilibrium; the number of times the generated χ^2 statistic exceeded the observed χ^2 statistic indicated the significance of the test on the observed data (Roff and Bentzen 1989). Data from multiple collections from different times or different sites within a river were compared using the heterogeneity log-likelihood ratio (G-test) both pairwise and simultaneously

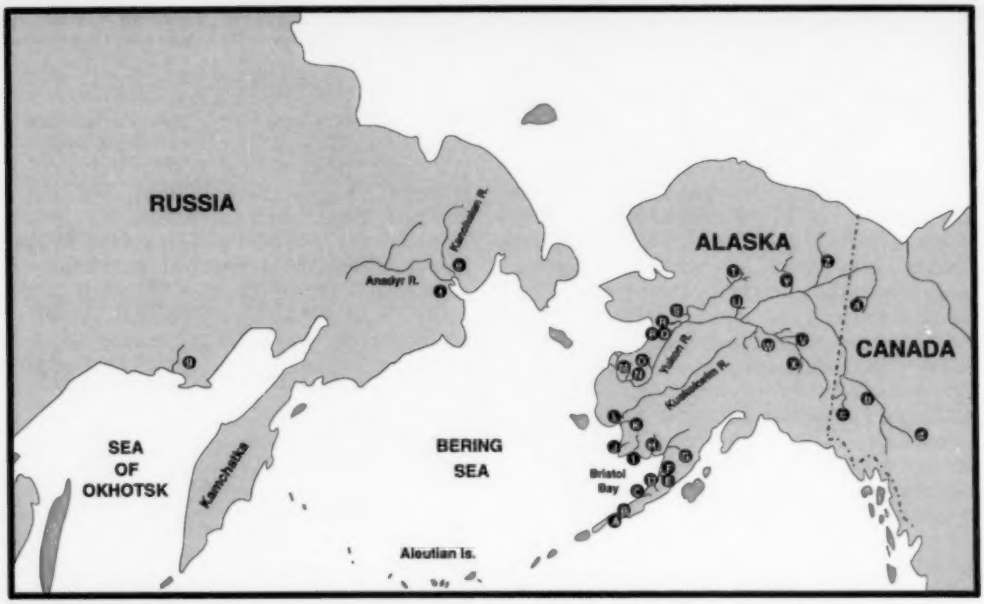

FIG. 1. Map of the North Pacific showing chum salmon sample sites in Russia, Alaska, and the Yukon Territory of Canada. Letters are identified in Table 1.

among groups (Sokal and Rohlf 1981). Collections from the same site made at different times, or collections from different locations within a river were pooled if they were not significantly different at the $P < 0.05$ level.

Chakraborty's (1980) gene diversity analysis was used to examine the hierarchial structure of genetic diversity. The total diversity was partitioned into hierarchial levels:

$$H_t = H_s + D_{sa} + D_{st} + D_{ac} + D_{ct}$$

where H_t = the total genetic diversity, H_s = the diversity within sites, D_{sa} = the diversity among sites within areas, D_{st} = the diversity among run timing with areas, D_{ac} = the diversity among areas within countries, D_{ct} = the diversity among countries.

Geographic patterns of genetic relatedness between populations were examined using Cavalli-Sforza and Edwards (1967) chord distance. Cluster analysis of the genetic distance data was done using the unweighted pair-group method (UPGMA; Sneath and Sokal 1973). Relatedness of populations was also examined using a principal components analysis of the arcsine transformation of gene frequencies using the PRINCOMP procedure from SAS (SAS Institute Inc. 1988). The neighbor-joining method of Saitou and Nei (1987) was used to construct a tree describing the relationship among stocks using the chord distance of Cavalli-Sforza and Edwards (1967).

Results

Of the 70 loci screened in the western Alaska and Russian chum salmon populations, variation was observed at 39, and 24 met the criteria for genetic analysis (Appendix 1). Four loci were found to be polymorphic in only one population: *FH** in the Kuskokwim River, *LDH-B1** in the Mother Goose collection, *LDH-C** in the Togiak River, and *PEPA** in the Alagnak River. Two loci were polymorphic in two collections: *mMDH-1** in the Goodnews and Kuskokwim rivers, and *TPI-1** in the 1987 collection from the Anvik River and the Nulato River. When tested for conformance to random mating (Hardy–Weinberg) proportions, 24 of 1224 tests were significantly different at the $P < 0.05$ level, less than the number expected by chance alone (61). No significant differences in allelic frequencies were observed among Yukon River fall-run chum salmon collections compared with results by Beacham et al.(1988) when using loci and stocks in common with this study. We found no significant differences in allelic frequencies among years in collections from the Fishing Branch River (2 yr), the Chandalar River (3 yr), the Sheenjek River (3 yr), the Delta River (2 yr), the Toklat River (2 yr), and the Salcha River (2 yr). We, therefore, pooled the multiple-year samples for these rivers. The two collections from the Anvik River (1987 and 1988) were significantly different ($G = 55.820$, df = 15, $P < 0.001$), and were, therefore, not pooled for the analysis. Multiple collections from within river systems within the same year were taken in the Nulato River (three sites), the Koyukuk River (four sites), the main stem of the Yukon River near Whitehorse, Yukon Territory (three sites), the Kuskokwim River (two sites), and the Goodnews River (2 sites). No significant differences were found in allelic frequencies among collections within rivers and were pooled prior to the analysis. The total number of populations used in the analysis was 33: 10 Yukon River summer run, 8 Yukon River fall run, 10 Bristol Bay area, 2 Alaska Peninsula, and 3 Russian.

Mean heterozygosity for the 10 Yukon River summer-run populations ranged from 0.058 to 0.072 ($\bar{x} = 0.064$), from 0.056 to 0.067 ($\bar{x} = 0.062$) for the eight Yukon River fall-run populations, from 0.056 to 0.072 ($\bar{x} = 0.065$) for the 10 Bristol Bay populations, and from 0.058 to 0.070 ($\bar{x} = 0.063$) for the three Russian populations. The percent polymorphic loci at the 0.01 level ranged from 33.33 to 35.19%

TABLE 1. Chum salmon sample sites, year, and number sampled. Letters in parentheses are used for identification in subsequent figures.

Population		Year	*N*	Population		Year	*N*
Yukon River (Alaska)				Yukon River (Canada)			
Summer run							
Andreafsky	(M)	1987	150	Fishing Branch	(a)	1987	129
Chulinak	(N)	1989	100			1989	50
Anvik	(O)	1987	150	Main Stem	(b)		
	(P)	1988	100	Big Creek		1987	70
Rodo	(Q)	1989	78	Minto		1989	100
Nulato	(R)			Tatchun		1987	75
Main Stem		1987	61	Kluane	(c)	1987	135
S. Fork		1987	71	Teslin	(d)	1989	95
N. Fork		1988	50	Bristol Bay area			
Gisasa	(S)	1989	97	Kuskokwim	(L)		
Koyukuk	(T)			Kwethluk		1989	77
Early		1990	75	Aniak		1989	75
Late		1990	75	Kanektok	(K)	1989	75
Henshaw		1987	43	Goodnews	(J)		
Jim		1987	101	Site 1		1989	50
Tozitna	(U)	1989	85	Site 2		1989	50
Salcha	(V)	1988	50	Togiak	(I)	1987	98
		1989	50	Nushagak	(H)	1987	87
Fall run							
Toklat	(W)	1987	135	Alagnak	(G)	1987	100
		1990	75	Naknek	(F)	1988	75
Delta	(X)	1987	135	Gertrude	(E)	1987	100
		1990	75	Mother Goose	(D)	1987	100
Bluff Cabin		1987	135	Meshik	(C)	1989	75
Chandalar	(Y)	1987	150	Alaska Peninsula			
		1988	73	Herendeen	(B)	1987	100
		1989	75	Nelson/Sapsuck	(A)	1987	100
Sheenjek	(Z)	1987	135	Russia			
		1988	80	Kanchalan	(e)	1991	80
		1989	80	Anadyr	(f)	1991	90
				Magadan	(g)	1991	80

($\bar{x}$ = 33.70%) for the Yukon River summer-run populations, from 27.78 to 33.33% ($\bar{x}$ = 31.25%) for the Yukon fall-run populations, from 29.63 to 37.04% ($\bar{x}$ = 32.25%) for the Bristol Bay populations, and from 29.63 to 31.48% ($\bar{x}$ = 30.86%) for the Russian populations.

The gene diversity analysis based on 24 polymorphic and 30 monomorphic loci shows that 95.42% of the diversity can be explained by heterogeneity within sites, 1.36% among sites within areas, 0.49% between run timings in the Yukon River populations, 1.69% among areas within countries, and 1.04% among countries (Table 3). *PEP-LT** was the largest contributor to the heterogeneity between the summer-run and fall-run fish in the Yukon River with *ESTD**, *bGLUA**, *sIDHP-2**, and *PEP-B2** also making substantial contributions. There was no differences between the two runs for *FH**, *LDH-B1**, *LDH-C**, *sMDH-1**, and *PEPA**. *ESTD** and *mIDHP-1** were by far the major contributors to the heterogeneity between the five major areas, with *sAAT-1,2**, *mAAT-1**, *LDH-A1**, and *mMEP-2** also contributing substantially. Only *FH**, *G3PDH-2**, and *TPI-1** were insignificant contributors to the heterogeneity among regions. *mIDHP-1** was the major factor in the heterogeneity between U.S. and Russian populations by a factor of almost three times more than the next largest contributor, ESTD*. Other important factors were *sAAT-1**, *mAAT-1,2**, *ALAT**, *sMDH-A1**, *PEP-LT**, *PGDH**, and *PEP-B2**. There was no difference between countries for *bGLUA**, *sMDH-1**, and *PEPA**.

The dendrogram (Fig. 2) shows three major groups: Yukon River, Bristol Bay area stocks, and a small group consisting of the two Alaska Peninsula stocks and the three Russian stocks. Within the Yukon River group, there is separation between the summer-run and fall-run stocks. The Kluane and Teslin river collections appear distinct from either group. Three collections from the south side of Bristol Bay, Meshik, Mother Goose, and Gertrude, form a separate group within the Bristol Bay area complex. The two Alaskan Peninsula stocks, Sapsuck and Herendeen, group more closely to the Russian stocks than to the more proximate Bristol Bay collections.

Two other methods were used to analyze the relationship among the collections. Principal components analysis using the arcsine transformation of the gene frequencies of the polymorphic loci displays the relationship in among the collections by graphing the first two components (Fig. 3). The first two components account for approximately 35% of the variability. The third component added another 15% but did not substantially add to the separation of groups. Twenty-one loci contributed substantially in the analysis. Only *FH** and *bGLUA** were insignificant contributors. Yukon River summer-run and fall-run collections are separated very well, as are the Russian and Alaska Peninsula collections. Principal

TABLE 2. Loci assayed in Western Alaska and Russian chum salmon showing IUBNC (1984) number, locus name (Shaklee et al. 1990b), tissue used, and buffer.

Enzyme name	Number	Locus	Tissue[a]	Buffer[b]
Polymorphic loci used in analysis				
Alanine aminotransferase	2.6.1.2	*ALAT*	H, M	EBT
Aspartate aminotransferase	2.6.1.1	*mAAT-1*	H, M	CAM 6.05, CAME 7.0
		sAAT-1,2	H, M	CAME 7.0, KG
Esterase-D	3.1.*.*	*ESTD*	H, M	KG, EBT
Fumarate hydratase	4.2.1.2	*FH**	L	CAME 7.0
N-Acetyl-β-glucosaminidase	3.2.1.30	*bGLUA*	L	CAME 7.0
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-2*	H, M	CAM 6.05, KG, CAME 7.0
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	*mIDHP-1*	H, M	CAME 7.0
		SIDHP-2	L	CAME 7.0
Lactate dehydrogenase	1.1.1.27	*LDH-A1*	M	KG
		LDH-B1	E, H	KG, EBT
		LDH-C	E	KG
Malate dehydrogenase	1.1.1.37	*sMDH-A1*	E, H, L	CAM 6.3, CAM 6.05
		sMDH-A2	E, H, L	CAM 6.3, CAM 6.05
		sMDH-B1, 2	E, H, L	CAM 6.3, KG, CAM 6.05
		mMDH-1	E, H, L	CAM 6.3, CAM 6.05
Malic enzyme ($NADP^+$)	1.1.1.40	*mMEP-2*	H, M	CAME 7.0, KG
Mannose-6-phosphate isomerase	5.3.1.8	*MPI*	E, H, L	EBT, KG, CAME 7.0, CAME 6.8
Tripeptide aminopeptidase	3.4.*.*	*PEPB-1*	H, L, M	EBT, CAM 6.05, KG
		PEPB-2	H, M	EBT, KG
Leucyl-tyrocine peptidase	3.4.*.*	*PEP-LT*	L, M	CAM 6.8, CAM 6.05, KG, CAME 7.0
Dipeptidase	3.4.*.*	*PEPA*	E, H, L	CAM 6.3, CAM 6.05, CAM 6.8
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH*	H, L, M	CAME 7.0
Triose-phosphate isomerase	5.3.1.1	*TPI-1*	E, H, M	KG, EBT
Polymorphic loci not used in analyses				
Aconitate hydratase	4.2.1.3	*mAH-3*	H	CAME 7.0
Aspartate aminotransferase	2.6.1.1	*sAAT-3*	E	EG
Creatine kinase	2.7.3.2	*CK-A1*	M	EBT
		CK-C2	E	EBT
Proline dipeptidase	3.4.13.9	*PEPD*	H	CAM 6.05
Formaldehyde dehydrogenase	1.2.1.1	*FGDH*	L	CAME 7.0, CAM 6.8
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B1,2*	H, M	EBT
Guanine deaminase	3.5.4.3	GDA	L	CAM 6.8
β-Galactosidase	3.2.1.23	*bGALA*	L	CAM 6.05
Phosphoglucomutase	5.4.2.2	*PGM-1*	H, L	EBT, CAM 6.8
		PGM-2	H, L, M	EBT, CAM 6.8, KG
		PGM-3,4	L	CAM 6.8
Triose-phosphate isomerase	5.3.1.1	*TPI-3*	E, H, M	KG, EBT
		TPI-4	E, H, M	KG, EBT
Gluatathione reductase	1.6.4.2	*GR*	E, H	KG
Monomorphic loci in all collections				
Aconitate hydratase	4.2.1.3	*mAH-1,2*	H	CAME 7.0
		mAH-4	H	CAME 7.0
		sAH	L	CAME 6.8
Acid phosphatase	3.1.3.2	*ACP*	L	CAME 7.0
Adenosine deaminase	3.5.4.4	*ADA-1*	E, H, L	CAM 6.3, CAME 7.0, CAM 6.05
		ADA-2	E, H	CAM 6.3, CAM 6.05, CAM 7.0
Aspartate aminotransferase	2.6.1.1	*sAAT-4*	L	CAM 6.05
Creatine kinase	2.7.3.2	*CK-A2*	M	EBT
		CK-C1	E	EBT
		CK-B	E	EBT
Diaphorase	1.6.*.*	*DIA*	H	KG
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1*	H, M	CAME 6.05, KG, CAME 7.0
		G3PHD-3	H, M	CAM 6.05, KG, CAME 7.0
		G3PHD-4	H, M	CAM 6.05, KG, CAME 7.0
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-4*	E	CAM 6.3
		GAPDH-5	E	CAM 6.3

TABLE 2. (*Concluded*).

Enzyme name	Number	Locus	Tissue[a]	Buffer[b]
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A*	E, H, M	EBT, CAM 6.3, EBT
Isocitrate dehydrogenase ($NADP^+$)	1.1.142	*mIDPH-2*	H, M	CAME 7.0
		sIDHP-1	L	CAME 7.0
Lactate dehydrogenase	1.1.1.27	*LDH-A2*	M	KG
		LDH-B2	E, H, L	KG, EBT
α-Mannosidase	3.2.1.24	*aMAN*	L	CAME 6.05
Malic enzyme ($NADP^+$)	1.1.1.40	*sMEP-1*	H, M	CAME 7.0, KG
Phosphoglycerate kinase	2.7.2.3	*PGK-1*	H, L	CAM 6.05, CAME 7.0
		PKG-2	H, L	CAM 6.05, CAME 7.0
Purine-nucleoside phosphorylase	2.4.2.1	*PNP*	E	CAM 6.3
Superoxide dismutase	1.15.1.1	*sSOD*	M	KG
Triose-phosphate isomerase	5.3.1.1	*TPI-2*	E, H, M	KG, EBT
α-Naphthyl propionate esterase	3.1.*.*	*EST*	L	CAM 6.05
Xanthine dehydrogenase	1.2.3.2	*XDH*	E	KG

[a]E = eye; M = heart; L = liver; M = muscle.
[b]Buffers and electrophoretic protocol are from Aebersold et al. (1987).

TABLE 3. Gene diversity analysis of western Alaska and Russian chum salmon populations based on 24 polymorphic and 30 monomorphic loci.

Source	Gene diversity		Coefficient of gene differentiation	
Average within sites	H_s	= 0.625	H_s/H_t	= 0.9542
Average among sites within areas	D_{sa}	= 0.0009	G_{sa}	= 0.0136
Average among run timing (Yukon River)	D_{st}	= 0.003	G_{st}	= 0.0049
Average among areas within countries	D_{ac}	= 0.0011	G_{ac}	= 0.0169
Among countries	D_{ct}	= 0.0007	G_{ct}	= 0.0104
Total gene diversity	H_t	= 0.0655		

components analysis shows what appears to be a very close relationship among Yukon River summer-run collections and those from Bristol Bay area. The two Yukon River fall-run collections (Kluane and Teslin rivers), which were difficult to assign to either run by analyzing the dendrogram, cluster with the other fall-run collections in the principal components analysis. The Kanchalan and Anadyr collections from Russia group very closely and are roughly equidistant from the Magadan, Herendeen, and southern Bristol Bay collections.

The neighbor-joining tree (Fig. 4) also shows the affinity between the Russian collections and the Alaska Peninsula collections. The three collections from the south end of Bristol Bay appear to form a distinct subgroup. Within the Yukon River summer run, the midriver collections from the Koyukuk, Tozitna, and Salcha rivers, form a distinct cluster. The structuring within the Yukon River fall-run collections is not as clear. The two midriver collections from the Delta and Toklat rivers form a group, and the four collections around the U.S.–Canada border form a group. Again the Kluane and Teslin rivers are distinct from the other fall-run collections.

Discussion

Substantial divergence was found among populations of chum salmon from western Alaska and the Russian Far East. In addition, further structuring was found within areas and within the Yukon River, particularly between the summer-run and fall-run populations. Extensive bootstrap simulations performed on the Yukon River chum salmon genetic baseline determined that the true proportion of summer-run and fall-run chum salmon in a mixture fell within within one standard deviation of the mean of the estimated proportion (Wilmot et al. 1992). Given the level of genetic divergence found among the four major areas in this study, determining the area of origin of fish caught in mixed-stock fisheries in the North Pacific Ocean should be relatively accurate and precise.

The observed stability of allele frequencies from year to year in the Yukon River collections supports results from

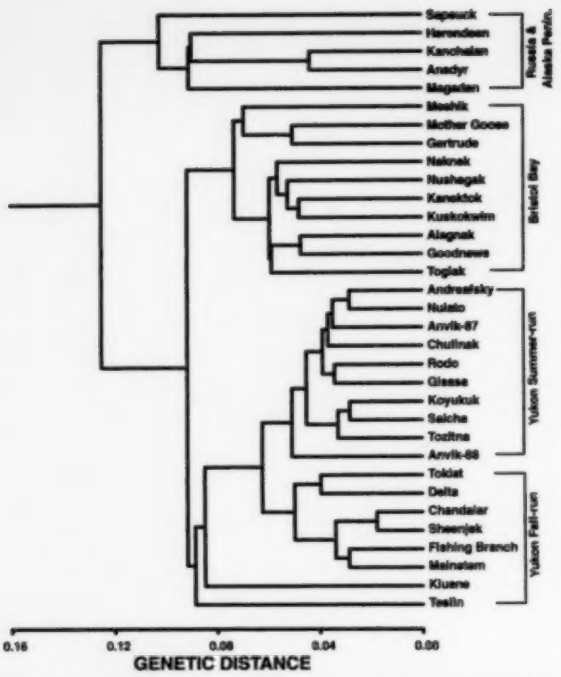

FIG. 2. UPGMA dendrogram showing the genetic relationship between populations of Russian and North American chum salmon using 24 polymorphic and 30 monomorphic loci. Genetic distance used is the Cavalli-Sforza and Edwards (1967) chord distance.

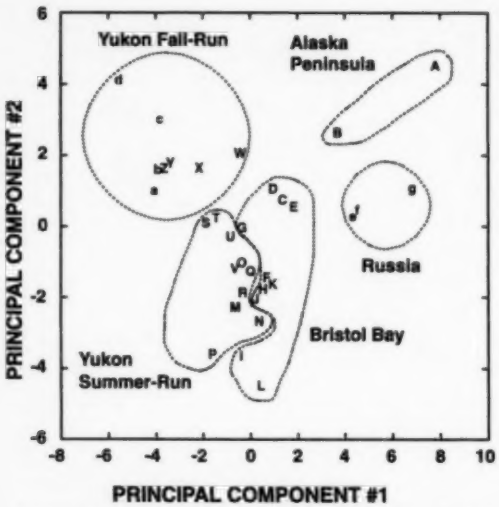

FIG. 3. Principal components analysis of Russian and North American chum salmon for 24 polymorphic loci. Letters are identified in Table 1.

other studies (Grant et al. 1980; Beacham et al. 1985; Beacham et al. 1988) and means that the data should accurately represent a population for an extended period of time. One exception was the significant difference between the samples from the Anvik River in 1987 and 1988. The Anvik River is a large, productive system with numerous spawning areas

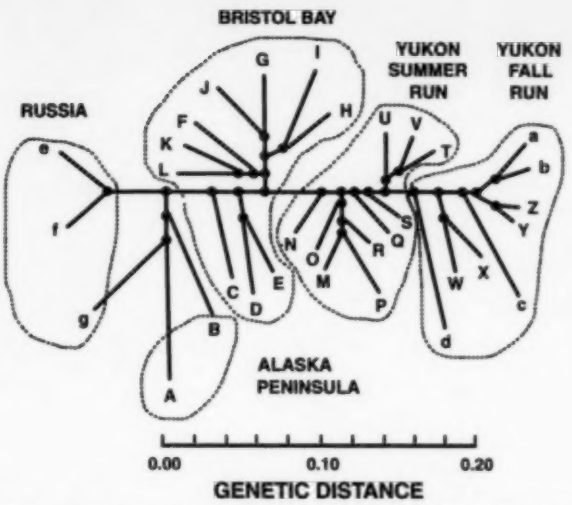

FIG. 4. Neighbor-joining tree (Saitou and Nei 1987) of Russian and North American chum salmon using the Cavalli-Sforza and Edwards (1967) chord distance using 24 polymorphic loci. Letters are identified in Table 1.

in headwater tributaries and the main stem. Our collections were made at a main stem sonar site and may be combinations of genetically distinct upriver stocks. The lack of a significant differences in allele frequencies among multiple collections within a tributary river shows there is little structuring at this level. However, this should be viewed with caution as we examined only a small portion of the total genome.

The gene diversity analysis revealed that approximately 1.0% of the variation found in the western Alaska and Russian chum salmon is due to differences among populations within geographic areas and 3.7% among areas within countries. The close affinity between the Russian and the Alaska Peninsula populations accounts for the fact that only 2.2% of the diversity is due to differences among Russian and western Alaskan populations. The substantial differences between the Yukon River summer-run and fall-run collections is not surprising because they are spatially and temporally separated.

The differences between the two Yukon River fall-run stocks (Kluane and Teslin), as compared with the other collections, are probably due to the complex glacial and postglacial history of these two watersheds. Most of the Yukon River drainage was ice free during the last great glaciation. The exception was the upper portion of the watershed in the Yukon Territory and British Columbia, which includes the Kluane and Teslin rivers. The geological history of the Kluane watershed has been studied by Lindsey et al. (1981). The Kluane Lake basin was probably completely covered by the McConnel ice advance. When the ice retreated, Kluane Lake may have been confluent with Glacial Lake Champagne. When Lake Champagne receded, Kluane Lake became a tributary to the Alsek River, which drains to the south. About 400 yr ago the Kaskawulsh Glacier advanced, cut off the flow to the Alsek River, and reversed the flow of the Kluane River towards the Yukon River through the White River. Biological evidence for this connection is shown by the presence of certain freshwater species in the Alsek River that are found in the Yukon drainage, but not in other river

systems draining south to the Gulf of Alaska. Whether the Kluane River chum salmon population came from the Alsek drainage, or is the result of a founder population from the Yukon River some 400 yr ago is unclear and awaits further genetics study of chum populations from rivers draining to the Gulf of Alaska. The Teslin River was also ice covered during the last glaciation, and may have a similar history of a connection to the south into the Taku River drainage (Kerr 1948).

The Bristol Bay area and the Alaska Peninsula were also covered during the maximum extent of the Wisconsinan and Illinoian glaciation (Pewe 1975). During this period, however, the central portion of Alaska was ice free, including the Yukon and Kuskokwim rivers. There is a close genetic affinity between the Lower Yukon River and Kuskokwim River chum salmon populations and the remainder of the southern coast and Bristol Bay. This suggests the Bristol Bay area was recolonized after the glaciers retreated by strays from the Lower Yukon and Kuskokwim rivers. The divergence of the Alaska Peninsula populations from the other western Alaskan populations, and their closer affinity with the Russian populations could be explained in two ways. First, we know Asian chum salmon migrate extensively through this area on their way to feeding grounds in the Gulf of Alaska and on their return to natal rivers. Strays from these Asian migrants could have colonized the area after the glacial retreat. The second alternative is that the area was colonized by a small founder stock from western Alaska and the genetic affinity with Russian stocks is strictly by chance.

We may soon have a clear picture of the distribution and genetic structure of chum salmon. Electrophoretic analysis of chum salmon populations is proceeding in a number of research laboratories, and results from populations throughout the range of chum salmon from Asia to North America will soon be available. Advances in DNA analytical techniques also promise to add another powerful tool to assist in unraveling the evolutionary past of the chum salmon.

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APPENDIX. Allelic frequencies at 24 polymorphic loci for chum salmon from western Alaska and Russia. Alleles are designated by their mobility relative to the most ommon allele (*100).

		*sAAT-1,2**				*mAAT-1**				*ALAT**			*ESTD**			*FH**			*G3PDH-2**				*bGLUA**			*mIDHP-1**		
Stock	Year	N	*100	*120	*84	N	*-100	*-120	*-70	N	*100	*93	N	*100	*120	N	*100	*75	N	*100	*93	*109	N	*100	*15	N	*100	*80
Yukon River (Summer)																												
Andreafsky	1987	150	0.923	0.077	0.000	37	0.946	0.000	0.054	149	0.866	0.134	150	0.547	0.453	150	1.000	0.000	150	0.880	0.120	0.000	83	0.922	0.078	149	0.983	0.017
Chulinak	1988	99	0.919	0.081	0.000	99	0.944	0.005	0.051	99	0.859	0.141	99	0.470	0.530	100	1.000	0.000	97	0.871	0.129	0.000	99	0.924	0.076	99	0.965	0.035
Anvik	1987	150	0.927	0.073	0.000	89	0.905	0.000	0.096	150	0.897	0.103	150	0.427	0.573	150	1.000	0.000	149	0.829	0.171	0.000	124	0.919	0.081	150	0.990	0.010
	1988	100	0.960	0.040	0.000	100	0.920	0.000	0.080	100	0.875	0.125	100	0.545	0.455	100	1.000	0.000	100	0.860	0.140	0.000	90	0.909	0.091	90	0.970	0.030
Rodo	1989	77	0.890	0.110	0.000	76	0.901	0.007	0.092	74	0.885	0.115	77	0.526	0.474	78	1.000	0.000	77	0.948	0.052	0.000	77	0.890	0.110	77	0.987	0.013
Nulato																												
Main stem	1987	61	0.918	0.082	0.000	60	0.933	0.000	0.067	61	0.885	0.115	61	0.500	0.500	61	1.000	0.000	61	0.869	0.131	0.000	58	0.922	0.078	61	0.975	0.025
South Fork	1987	71	0.887	0.113	0.000	65	0.939	0.000	0.062	71	0.887	0.113	71	0.472	0.528	71	1.000	0.000	71	0.859	0.141	0.000	69	0.906	0.094	71	0.979	0.021
North Fork	1988	50	0.880	0.120	0.000	50	0.900	0.000	0.100	50	0.850	0.150	50	0.490	0.510	50	1.000	0.000	50	0.870	0.130	0.000	50	0.900	0.100	50	0.980	0.020
	Pooled	182	0.896	0.104	0.000	175	0.926	0.000	0.074	182	0.876	0.124	182	0.486	0.514	182	1.000	0.000	182	0.865	0.135	0.000	177	0.910	0.090	182	0.978	0.022
Gisasa	1989	97	0.912	0.088	0.000	97	0.923	0.000	0.077	96	0.932	0.068	94	0.521	0.479	97	1.000	0.000	91	0.929	0.071	0.000	97	0.928	0.072	97	0.990	0.010
Koyukuk																												
Henshaw	1987	43	0.942	0.058	0.000	43	0.849	0.000	0.151	43	0.930	0.070	43	0.477	0.523	43	1.000	0.000	43	0.872	0.128	0.000	38	0.921	0.079	43	0.965	0.035
Jim	1987	101	0.871	0.129	0.000	101	0.866	0.000	0.134	90	0.900	0.100	100	0.440	0.560	101	1.000	0.000	101	0.891	0.109	0.000	84	0.936	0.066	101	0.960	0.040
So. Fork, Early	1990	74	0.878	0.122	0.000	74	0.899	0.041	0.061	74	0.953	0.047	74	0.453	0.547	74	1.000	0.000	73	0.932	0.069	0.000	73	0.932	0.069	74	0.953	0.047
So. Fork, Late	1990	75	0.927	0.073	0.000	75	0.893	0.000	0.107	75	0.947	0.053	75	0.420	0.580	75	1.000	0.000	75	0.893	0.107	0.000	75	0.927	0.073	75	0.980	0.020
	Pooled	293	0.898	0.102	0.000	293	0.879	0.010	0.111	282	0.931	0.069	292	0.443	0.557	293	1.000	0.000	292	0.899	0.101	0.000	270	0.930	0.070	293	0.964	0.036
Tozitna	1989	85	0.900	0.100	0.000	85	0.865	0.000	0.135	84	0.935	0.066	85	0.512	0.488	85	1.000	0.000	85	0.882	0.118	0.000	77	0.935	0.065	85	0.977	0.024
Salcha	1988	48	0.958	0.042	0.000	48	0.896	0.000	0.104	50	0.950	0.050	48	0.510	0.490	48	1.000	0.000	48	0.813	0.188	0.000	49	0.959	0.041	49	0.927	0.073
	1989	50	0.880	0.120	0.000	50	0.920	0.010	0.070	50	0.950	0.050	50	0.460	0.540	50	1.000	0.000	50	0.940	0.060	0.000	50	0.930	0.070	50	0.960	0.040
	Pooled	98	0.918	0.082	0.000	98	0.908	0.005	0.087	100	0.950	0.050	98	0.485	0.515	98	1.000	0.000	98	0.878	0.122	0.000	99	0.944	0.056	98	0.944	0.056
Yukon River (Fall)																												
Toklat	1987	133	0.850	0.150	0.000	69	0.928	0.000	0.073	132	0.845	0.155	135	0.270	0.730	135	1.000	0.000	134	0.787	0.213	0.000	75	0.920	0.080	135	0.956	0.044
	1990	74	0.858	0.142	0.000	74	0.892	0.000	0.108	74	0.912	0.088	74	0.284	0.716	74	1.000	0.000	73	0.836	0.164	0.000	73	0.945	0.055	74	0.953	0.047
	Pooled	207	0.853	0.147	0.000	143	0.909	0.000	0.091	206	0.869	0.131	209	0.275	0.725	209	1.000	0.000	207	0.804	0.196	0.000	148	0.932	0.068	209	0.955	0.046
Delta	1987	135	0.878	0.122	0.000	129	0.919	0.004	0.078	134	0.937	0.063	135	0.196	0.804	135	1.000	0.000	135	0.863	0.137	0.000	117	0.966	0.034	135	0.982	0.018
	1990	74	0.858	0.142	0.000	74	0.892	0.000	0.108	74	0.912	0.088	75	0.167	0.833	75	1.000	0.000	75	0.840	0.160	0.000	74	0.932	0.068	75	0.987	0.013
Bluff Cabin	1987	135	0.878	0.122	0.000	71	0.937	0.000	0.063	133	0.921	0.079	134	0.198	0.802	135	1.000	0.000	134	0.843	0.157	0.000	82	0.933	0.067	135	0.985	0.015
	Pooled	345	0.880	0.120	0.000	275	0.935	0.002	0.064	343	0.927	0.072	344	0.190	0.810	345	1.000	0.000	345	0.850	0.150	0.000	273	0.947	0.053	345	0.984	0.016
Chandalar	1987	140	0.913	0.087	0.000	148	0.878	0.000	0.122	28	0.911	0.089	145	0.341	0.659	145	1.000	0.000	148	0.865	0.135	0.000	52	0.962	0.039	149	0.990	0.010
	1988	73	0.897	0.103	0.000	73	0.877	0.000	0.123	73	0.918	0.082	73	0.308	0.692	73	1.000	0.000	73	0.849	0.151	0.000	71	0.916	0.085	73	0.993	0.007
	1989	78	0.867	0.133	0.000	75	0.907	0.000	0.093	75	0.947	0.053	75	0.327	0.673	75	1.000	0.000	75	0.900	0.100	0.000	75	0.907	0.093	75	0.993	0.007
	Pooled	297	0.897	0.103	0.000	296	0.895	0.000	0.115	176	0.929	0.071	293	0.329	0.671	297	1.000	0.000	296	0.870	0.130	0.000	198	0.924	0.076	297	0.992	0.008
Sheenjek	1987	135	0.904	0.096	0.000	79	0.911	0.000	0.089	135	0.941	0.059	135	0.344	0.656	135	1.000	0.000	134	0.881	0.119	0.000	32	0.969	0.031	135	0.982	0.019
	1988	79	0.905	0.095	0.000	78	0.917	0.000	0.083	80	0.938	0.063	79	0.335	0.665	79	1.000	0.000	78	0.891	0.109	0.000	80	0.881	0.119	79	1.000	0.000
	1989	80	0.888	0.113	0.000	80	0.863	0.000	0.138	80	0.913	0.088	80	0.400	0.600	80	1.000	0.000	80	0.863	0.138	0.000	80	0.913	0.088	80	0.981	0.019
	Pooled	294	0.900	0.100	0.000	237	0.897	0.000	0.103	295	0.932	0.068	294	0.357	0.643	295	1.000	0.000	292	0.878	0.122	0.000	192	0.909	0.091	294	0.986	0.014
Fishing Branch	1987	128	0.938	0.063	0.000	73	0.863	0.007	0.130	128	0.941	0.059	129	0.252	0.748	129	1.000	0.000	128	0.848	0.152	0.000	30	0.983	0.017	129	0.996	0.004
	1989	49	0.918	0.082	0.000	49	0.878	0.000	0.122	49	0.949	0.051	49	0.388	0.612	49	1.000	0.000	49	0.847	0.153	0.000	48	0.938	0.063	49	1.000	0.000
	Pooled	177	0.932	0.068	0.000	122	0.869	0.004	0.127	177	0.944	0.057	178	0.289	0.711	178	1.000	0.000	177	0.848	0.153	0.000	78	0.955	0.045	178	0.997	0.003
Main Stem																												
Big Creek	1987	69	0.877	0.123	0.000	37	0.960	0.000	0.041	69	0.935	0.065	69	0.283	0.717	60	1.000	0.000	69	0.891	0.109	0.000	31	0.968	0.032	69	1.000	0.000
Tatchun	1987	75	0.893	0.107	0.000	59	0.898	0.000	0.102	75	0.927	0.073	75	0.320	0.680	75	1.000	0.000	74	0.892	0.108	0.000	24	0.979	0.021	75	1.000	0.000
Minto	1989	100	0.930	0.070	0.000	100	0.920	0.000	0.080	100	0.955	0.045	100	0.345	0.655	100	1.000	0.000	100	0.875	0.125	0.000	100	0.965	0.035	100	1.000	0.000
	Pooled	244	0.904	0.096	0.000	196	0.921	0.000	0.079	244	0.941	0.059	244	0.320	0.680	244	1.000	0.000	243	0.885	0.115	0.000	100	0.968	0.032	244	1.000	0.000
Kluane	1987	133	0.895	0.105	0.000	66	0.909	0.000	0.091	143	0.969	0.031	143	0.255	0.745	150	1.000	0.000	134	0.828	0.172	0.000	31	0.774	0.226	135	1.000	0.000
Teslin	1989	95	0.905	0.095	0.000	95	0.868	0.005	0.126	95	0.963	0.037	95	0.568	0.432	95	1.000	0.000	94	0.973	0.027	0.000	93	0.957	0.043	95	1.000	0.000
Bristol Bay Area																												
Kuskokwim																												
Kwethlok	1989	77	0.942	0.058	0.000	77	0.935	0.065	0.000	77	0.857	0.143	75	0.493	0.507	75	1.000	0.000	75	0.820	0.180	0.000	75	0.967	0.033	77	0.987	0.013
Aniak	1989	75	0.953	0.047	0.000	75	0.933	0.067	0.000	75	0.860	0.140	75	0.500	0.500	75	0.993	0.007	75	0.833	0.167	0.000	74	0.946	0.054	75	0.973	0.027
	Pooled	152	0.947	0.053	0.000	152	0.934	0.066	0.000	152	0.859	0.141	152	0.513	0.487	152	0.993	0.007	152	0.829	0.171	0.000	152	0.914	0.086	152	0.980	0.020
Kanektok	1989	75	0.873	0.127	0.000	75	0.907	0.093	0.000	75	0.880	0.120	75	0.493	0.507	75	1.000	0.000	75	0.820	0.180	0.000	75	0.913	0.087	75	0.993	0.007
Goodnews	1989	50	0.960	0.040	0.000	50	0.880	0.120	0.000	50	0.930	0.070	49	0.561	0.439	50	1.000	0.000	50	0.890	0.110	0.000	50	1.000	0.000	50	0.970	0.030
	1989	50	0.900	0.100	0.000	50	0.910	0.090	0.000	50	0.940	0.060	50	0.510	0.490	50	1.000	0.000	49	0.898	0.102	0.000	11	0.955	0.045	50	0.990	0.010
	Pooled	100	0.930	0.070	0.000	100	0.895	0.105	0.000	100	0.935	0.065	99	0.535	0.465	100	1.000	0.000	99	0.894	0.106	0.000	99	0.929	0.071	100	0.980	0.020
Togiak	1987	98	0.913	0.087	0.000	58	0.845	0.155	0.000	45	0.944	0.056	98	0.526	0.474	98	1.000	0.000	98	0.852	0.148	0.000	21	0.976	0.024	98	0.985	0.015
Nushagak	1987/88	87	0.887	0.098	0.006	87	0.891	0.109	0.000	88	0.895	0.105	87	0.494	0.506	87	1.000	0.000	87	0.862	0.138	0.000	77	0.981	0.019	87	0.977	0.023
Alagnak	1987	98	0.903	0.097	0.000	72	0.924	0.076	0.000	36	0.958	0.042	98	0.633	0.367	98	1.000	0.000	96	0.953	0.047	0.000	22	0.909	0.091	98	0.959	0.041
Naknek	1988	74	0.899	0.101	0.000	74	0.926	0.074	0.000	74	0.824	0.176	74	0.541	0.459	74	1.000	0.000	74	0.885	0.115	0.000	73	0.986	0.034	74	0.986	0.014
Gertrude	1987	100	0.840	0.150	0.010	71	0.880	0.113	0.007	65	0.877	0.123	99	0.566	0.434	100	1.000	0.000	100	0.930	0.070	0.000	41	0.927	0.073	100	0.910	0.090
Mother Goose	1987	100	0.870	0.115	0.015	54	0.898	0.102	0.000	93	0.882	0.118	100	0.505	0.495	100	1.000	0.000	100	0.945	0.055	0.000	19	1.000	0.000	100	0.915	0.085
Meshik	1989	75	0.907	0.093	0.000	75	0.840	0.153	0.007	75	0.860	0.140	75	0.567	0.433	75	1.000	0.000	75	0.867	0.133	0.000	75	0.960	0.040	75	0.893	0.107
Herendeen	1987	100	0.750	0.215	0.035	64	0.883	0.055	0.063	69	0.855	0.145	100	0.915	0.085	100	1.000	0.000	100	0.870	0.130	0.000	23	0.891	0.109	100	0.800	0.200
Nelson/Sapsuck	1987	99	0.753	0.217	0.030	54	0.944	0.046	0.009	36	0.861	0.139	100	0.885	0.115	100	1.000	0.000	100	0.840	0.160	0.000	19	0.947	0.053	100	0.940	0.060
Russia																												
Magaden	1991	80	0.725	0.275	0.000	78	0.994	0.006	0.000	60	0.725	0.275	80	0.913	0.087	80	1.000	0.000	78	0.872	0.122	0.006	77	0.942	0.058	80	0.906	0.094
Anadyr	1991	80	0.856	0.144	0.000	79	0.962	0.000	0.038	80	0.794	0.206	80	0.712	0.287	80	1.000	0.000	79	0.854	0.133	0.013	80	0.938	0.063	80	0.575	0.425
Kanchalan	1991	78	0.872	0.128	0.000	78	0.968	0.013	0.019	61	0.877	0.123	67	0.761	0.239	79	1.000	0.000	78	0.910	0.090	0.000	76	0.901	0.099	78	0.559	0.441

APPENDIX (*Continued*).

		sIDHP-2*								LDH-A1*			LDH-B1*			LDH-C*			sMDH-A1*			sMDH-A2*			sMDH-B1,2*					
Stock	Year	N	*100	*35	*85	*25	*65	*110	*51	N	*-100	*50	N	*100	*45	N	*100	*95	N	*100	*200	N	*100	*40	N	*100	*72	*130	*50	*145
Yukon River (Summer)																														
Andreafsky	1987	150	0.450	0.487	0.063	0.000	0.000	0.000	0.000	150	0.827	0.173	150	1.000	0.000	150	1.000	0.000	150	0.947	0.053	149	0.963	0.037	150	0.993	0.007	0.000	0.000	0.000
Chulinak	1989	97	0.428	0.521	0.041	0.010	0.000	0.000	0.000	99	0.793	0.207	100	1.000	0.000	100	1.000	0.000	98	0.954	0.046	99	0.985	0.015	99	0.975	0.025	0.000	0.000	0.000
Anvik	1987	149	0.453	0.483	0.064	0.000	0.000	0.000	0.000	150	0.840	0.160	150	1.000	0.000	150	1.000	0.000	150	0.960	0.040	150	0.990	0.010	150	0.987	0.010	0.003	0.000	0.000
	1988	100	0.500	0.460	0.035	0.005	0.000	0.000	0.000	100	0.750	0.250	100	1.000	0.000	100	1.000	0.000	99	0.894	0.106	50	0.980	0.020	100	0.980	0.010	0.010	0.000	0.000
Rodo	1989	77	0.435	0.487	0.078	0.000	0.000	0.000	0.000	77	0.825	0.175	78	1.000	0.000	78	1.000	0.000	77	0.935	0.065	77	0.987	0.013	77	0.981	0.020	0.000	0.000	0.000
Nulato																														
Main stem	1987	61	0.500	0.459	0.041	0.000	0.000	0.000	0.000	61	0.795	0.205	61	1.000	0.000	61	1.000	0.000	61	0.934	0.066	61	1.000	0.000	61	0.992	0.008	0.000	0.000	0.000
South Fork	1987	71	0.486	0.430	0.085	0.000	0.000	0.000	0.000	71	0.711	0.289	71	1.000	0.000	71	1.000	0.000	71	0.951	0.035	71	0.951	0.049	71	0.979	0.021	0.000	0.000	0.000
North Fork	1988	50	0.460	0.480	0.060	0.000	0.000	0.000	0.000	50	0.690	0.310	50	1.000	0.000	50	1.000	0.000	50	0.940	0.060	50	0.970	0.030	50	0.990	0.000	0.010	0.000	0.000
	Pooled	182	0.484	0.453	0.063	0.000	0.000	0.000	0.000	182	0.734	0.267	182	1.000	0.000	182	1.000	0.000	182	0.948	0.052	182	0.973	0.027	182	0.986	0.011	0.003	0.000	0.000
Gisasa	1989	97	0.485	0.454	0.062	0.000	0.000	0.000	0.000	97	0.768	0.232	97	1.000	0.000	97	1.000	0.000	97	0.902	0.098	97	0.969	0.031	97	0.990	0.010	0.000	0.000	0.000
Koyukuk																														
Henshaw	1987	43	0.500	0.430	0.070	0.000	0.000	0.000	0.000	43	0.802	0.198	43	1.000	0.000	43	1.000	0.000	43	0.919	0.081	43	0.977	0.023	43	0.977	0.023	0.000	0.000	0.000
Jim	1987	101	0.500	0.470	0.030	0.000	0.000	0.000	0.000	101	0.753	0.248	101	1.000	0.000	101	1.000	0.000	101	0.886	0.114	101	0.946	0.055	101	0.946	0.045	0.010	0.000	0.000
So. Fork, Early	1990	74	0.554	0.378	0.068	0.000	0.000	0.000	0.000	74	0.737	0.264	74	1.000	0.000	74	1.000	0.000	74	0.939	0.061	74	1.000	0.000	74	0.987	0.014	0.000	0.000	0.000
So. Fork, Late	1990	75	0.533	0.400	0.067	0.000	0.000	0.000	0.000	75	0.807	0.193	75	1.000	0.000	75	1.000	0.000	75	0.933	0.067	75	0.993	0.007	75	0.987	0.013	0.000	0.000	0.000
	Pooled	293	0.522	0.423	0.055	0.000	0.000	0.000	0.000	293	0.770	0.230	293	1.000	0.000	293	1.000	0.000	293	0.918	0.084	293	0.976	0.024	293	0.971	0.026	0.003	0.000	0.000
Tozitna	1989	85	0.524	0.400	0.071	0.006	0.000	0.000	0.000	85	0.771	0.229	85	1.000	0.000	85	1.000	0.000	85	0.959	0.041	85	0.965	0.035	85	0.959	0.041	0.000	0.000	0.000
Salcha	1988	48	0.521	0.448	0.031	0.000	0.000	0.000	0.000	48	0.719	0.281	48	1.000	0.000	48	1.000	0.000	48	0.969	0.031	48	1.000	0.000	48	0.958	0.042	0.000	0.000	0.000
	1989	50	0.530	0.400	0.070	0.000	0.000	0.000	0.000	50	0.790	0.210	50	1.000	0.000	50	1.000	0.000	50	0.950	0.050	48	0.990	0.010	50	0.950	0.040	0.010	0.000	0.000
	Pooled	98	0.526	0.424	0.051	0.000	0.000	0.000	0.000	98	0.755	0.245	98	1.000	0.000	98	1.000	0.000	98	0.959	0.041	96	0.995	0.005	98	0.954	0.041	0.005	0.000	0.000
Yukon River (Fall)																														
Tolstat	1987	135	0.633	0.304	0.063	0.000	0.000	0.000	0.000	135	0.756	0.244	135	1.000	0.000	135	1.000	0.000	135	0.930	0.070	135	0.978	0.022	135	0.985	0.015	0.000	0.000	0.000
	1990	74	0.615	0.338	0.047	0.000	0.000	0.000	0.000	72	0.757	0.243	74	1.000	0.000	74	1.000	0.000	74	0.939	0.061	74	0.987	0.014	74	1.000	0.000	0.000	0.000	0.000
	Pooled	209	0.627	0.316	0.057	0.000	0.000	0.000	0.000	207	0.756	0.244	209	1.000	0.000	209	1.000	0.000	209	0.933	0.067	209	0.981	0.019	209	0.990	0.010	0.000	0.000	0.000
Delta	1987	135	0.533	0.407	0.059	0.000	0.000	0.000	0.000	135	0.704	0.296	135	1.000	0.000	135	1.000	0.000	130	0.939	0.062	135	0.993	0.007	135	1.000	0.000	0.000	0.000	0.000
	1990	75	0.540	0.440	0.020	0.000	0.000	0.000	0.000	74	0.669	0.331	75	1.000	0.000	75	1.000	0.000	75	0.933	0.067	75	0.973	0.027	75	1.000	0.000	0.000	0.000	0.000
Stuff Cabin	1987	135	0.556	0.385	0.059	0.000	0.000	0.000	0.000	135	0.656	0.344	135	1.000	0.000	135	1.000	0.000	135	0.944	0.056	135	0.989	0.011	135	1.000	0.000	0.000	0.000	0.000
	Pooled	345	0.543	0.408	0.051	0.000	0.000	0.000	0.000	344	0.677	0.323	345	1.000	0.000	345	1.000	0.000	340	0.940	0.060	345	0.987	0.013	345	1.000	0.000	0.000	0.000	0.000
Chandalar	1987	149	0.416	0.544	0.040	0.000	0.000	0.000	0.000	149	0.711	0.289	149	1.000	0.000	149	1.000	0.000	149	0.826	0.175	149	0.983	0.017	149	0.990	0.010	0.000	0.000	0.000
	1988	73	0.390	0.569	0.041	0.000	0.000	0.000	0.000	72	0.792	0.208	73	1.000	0.000	73	1.000	0.000	73	0.918	0.082	73	0.980	0.021	73	0.993	0.007	0.000	0.000	0.000
	1989	75	0.440	0.513	0.047	0.000	0.000	0.000	0.000	75	0.673	0.327	75	1.000	0.000	75	1.000	0.000	75	0.907	0.093	75	0.967	0.033	75	1.000	0.000	0.000	0.000	0.000
	Pooled	297	0.416	0.542	0.042	0.000	0.000	0.000	0.000	296	0.721	0.279	298	1.000	0.000	298	1.000	0.000	297	0.869	0.131	297	0.978	0.022	297	0.993	0.070	0.000	0.000	0.000
Sheenjek	1987	135	0.400	0.563	0.037	0.000	0.000	0.000	0.000	135	0.696	0.304	135	1.000	0.000	135	1.000	0.000	135	0.870	0.130	135	0.970	0.030	135	1.000	0.000	0.000	0.000	0.000
	1988	78	0.314	0.628	0.058	0.000	0.000	0.000	0.000	77	0.780	0.240	79	1.000	0.000	79	1.000	0.000	79	0.854	0.146	79	0.958	0.044	79	1.000	0.000	0.000	0.000	0.000
	1989	80	0.381	0.550	0.069	0.000	0.000	0.000	0.000	80	0.650	0.350	80	1.000	0.000	80	1.000	0.000	80	0.900	0.100	80	0.981	0.019	80	0.994	0.006	0.000	0.000	0.000
	Pooled	293	0.372	0.577	0.051	0.000	0.000	0.000	0.000	292	0.700	0.300	294	1.000	0.000	294	1.000	0.000	294	0.874	0.126	294	0.969	0.031	294	0.998	0.002	0.000	0.000	0.000
Fishing Branch	1987	122	0.414	0.557	0.029	0.000	0.000	0.000	0.000	129	0.632	0.368	129	1.000	0.000	129	1.000	0.000	129	0.930	0.070	126	0.968	0.032	129	1.000	0.000	0.000	0.000	0.000
	1989	49	0.357	0.602	0.041	0.000	0.000	0.000	0.000	49	0.643	0.357	49	1.000	0.000	49	1.000	0.000	49	0.929	0.071	49	0.980	0.010	49	1.000	0.000	0.000	0.000	0.000
	Pooled	171	0.398	0.570	0.032	0.000	0.000	0.000	0.000	178	0.635	0.365	178	1.000	0.000	178	1.000	0.000	178	0.930	0.070	175	0.974	0.026	178	1.000	0.000	0.000	0.000	0.000
Main Stem																														
Big Creek	1987	69	0.406	0.573	0.022	0.000	0.000	0.000	0.000	69	0.696	0.304	69	1.000	0.000	69	1.000	0.000	69	0.891	0.109	69	0.957	0.044	69	1.000	0.000	0.000	0.000	0.000
Tetchun	1987	73	0.452	0.507	0.041	0.000	0.000	0.000	0.000	75	0.713	0.287	75	1.000	0.000	75	1.000	0.000	71	0.866	0.134	75	0.933	0.067	75	1.000	0.000	0.000	0.000	0.000
Minto	1989	100	0.475	0.485	0.040	0.000	0.000	0.000	0.000	100	0.710	0.290	100	1.000	0.000	100	1.000	0.000	100	0.915	0.085	100	0.990	0.010	100	1.000	0.000	0.000	0.000	0.000
	Pooled	242	0.448	0.517	0.035	0.000	0.000	0.000	0.000	244	0.707	0.293	244	1.000	0.000	244	1.000	0.000	240	0.894	0.106	244	0.963	0.037	244	1.000	0.000	0.000	0.000	0.000
Kuskokwim	1987	135	0.344	0.596	0.059	0.000	0.000	0.000	0.000	135	0.533	0.467	150	1.000	0.000	150	1.000	0.000	135	0.948	0.052	134	0.985	0.015	135	0.996	0.004	0.000	0.000	0.000
Teslin	1989	95	0.626	0.337	0.037	0.000	0.000	0.000	0.000	95	0.700	0.300	95	1.000	0.000	95	1.000	0.000	86	0.842	0.158	94	0.957	0.043	95	1.000	0.000	0.000	0.000	0.000
Bristol Bay Area																														
Kuskokwim																														
Kwethlok	1989	77	0.500	0.461	0.026	0.013	0.000	0.000	0.000	77	0.773	0.227	77	1.000	0.000	77	1.000	0.000	77	0.955	0.045	77	1.000	0.000	77	0.981	0.000	0.000	0.006	0.013
Aniak	1989	75	0.413	0.500	0.073	0.013	0.000	0.000	0.000	75	0.840	0.160	75	1.000	0.000	75	1.000	0.000	75	0.967	0.033	75	0.980	0.020	75	0.993	0.007	0.000	0.000	0.000
	Pooled	152	0.457	0.480	0.049	0.013	0.000	0.000	0.000	152	0.806	0.194	152	1.000	0.000	152	1.000	0.000	152	0.961	0.039	152	0.990	0.010	152	0.987	0.003	0.000	0.003	0.007
Kanektok	1989	75	0.433	0.473	0.073	0.020	0.000	0.000	0.000	75	0.760	0.240	75	1.000	0.000	75	1.000	0.000	75	0.933	0.067	75	0.973	0.027	75	0.967	0.033	0.000	0.000	0.000
Goodnews	1988	50	0.450	0.430	0.120	0.000	0.000	0.000	0.000	50	0.840	0.160	50	1.000	0.000	50	1.000	0.000	50	0.970	0.030	50	1.000	0.000	50	0.990	0.000	0.010	0.000	0.000
	1989	50	0.430	0.480	0.090	0.000	0.000	0.000	0.000	50	0.720	0.280	50	1.000	0.000	50	1.000	0.000	50	0.930	0.070	50	1.000	0.000	50	0.980	0.000	0.010	0.010	0.000
	Pooled	100	0.440	0.455	0.105	0.000	0.000	0.000	0.000	100	0.780	0.220	100	1.000	0.000	100	1.000	0.000	100	0.950	0.050	100	1.000	0.000	100	0.985	0.000	0.010	0.005	0.000
Togiak	1987	98	0.495	0.490	0.015	0.000	0.000	0.000	0.000	73	0.897	0.103	98	1.000	0.000	98	0.985	0.015	98	0.954	0.046	98	1.000	0.000	98	0.969	0.026	0.000	0.005	0.000
Nushagak	1987/88	87	0.437	0.448	0.103	0.011	0.000	0.000	0.000	87	0.787	0.213	87	1.000	0.000	87	1.000	0.000	87	0.960	0.040	87	1.000	0.000	87	0.997	0.023	0.000	0.000	0.000
Alagnak	1987	98	0.464	0.459	0.066	0.010	0.000	0.000	0.000	98	0.806	0.194	98	1.000	0.000	98	1.000	0.000	97	0.943	0.057	76	1.000	0.000	98	0.995	0.005	0.000	0.000	0.000
Naknek	1988	74	0.500	0.466	0.034	0.000	0.000	0.000	0.000	74	0.878	0.122	74	1.000	0.000	74	1.000	0.000	73	0.952	0.048	74	0.980	0.020	74	0.993	0.000	0.000	0.000	7.000
Gertrude	1987	99	0.505	0.369	0.066	0.061	0.000	0.000	0.000	100	0.710	0.290	100	1.000	0.000	100	1.000	0.000	73	0.938	0.062	35	1.000	0.000	100	0.975	0.000	0.025	0.000	0.000
Mother Goose	1987	100	0.425	0.480	0.075	0.040	0.000	0.000	0.000	100	0.750	0.250	100	0.995	0.005	100	1.000	0.000	100	0.945	0.055	61	1.000	0.250	0	0.990	0.000	0.005	0.005	0.000
Meshik	1989	75	0.433	0.393	0.093	0.080	0.000	0.000	0.000	75	0.933	0.067	75	1.000	0.000	75	0.867	0.133	75	0.880	0.120	75	1.000	0.000	75	0.983	0.000	0.000	0.007	0.000
Herendeen	1987	89	0.424	0.439	0.081	0.076	0.000	0.000	0.000	100	0.900	0.100	100	1.000	0.000	100	1.000	0.000	100	0.890	0.110	100	0.995	0.005	100	0.945	0.005	0.025	0.015	0.010
Nelson/Sapeuck	1987	100	0.365	0.290	0.245	0.195	0.005	0.000	0.000	100	0.975	0.025	100	1.000	0.000	100	1.000	0.000	94	0.989	0.011	91	0.989	0.011	100	0.925	0.050	0.075	0.000	0.000
Russia																														
Magaden	1991	78	0.596	0.250	0.071	0.071	0.000	0.000	0.013	79	0.911	0.089	80	1.000	0.000	78	1.000	0.000	80	0.981	0.019	80	0.994	0.006	80	0.969	0.013	0.000	0.000	0.000
Anadyr	1991	80	0.381	0.469	0.094	0.044	0.000	0.006	0.006	80	0.869	0.131	78	1.000	0.000	66	1.000	0.000	80	0.988	0.013	80	1.000	0.000	80	0.969	0.013	0.000	0.000	0.000
Kanchalan	1991	78	0.391	0.442	0.109	0.045	0.000	0.000	0.013	76	0.934	0.066	79	1.000	0.000	72	1.000	0.000	78	0.981	0.019	79	0.994	0.006	78	0.994	0.006	0.000	0.000	0.000

APPENDIX (*Concluded*).

Stock	Year	mMDH-1*			mMEP-2*			MPI			PEPA			PEP-LT*			PGDH*				PEPB-1*				PEPB-2*			TPI-1*		
		N	*100	*50	N	*100	*122	N	*100	*97	N	*100	*110	N	*100	*85	N	*100	*92	*104	N	*-100	*-156	*-128	*-127	N	*100	*132	N	*-100	*-60
Yukon River (Summer)																														
Andreafsky	1987	150	1.000	0.000	150	0.807	0.193	150	0.837	0.163	146	1.000	0.000	46	0.957	0.004	148	0.956	0.045	0.000	150	0.943	0.043	0.013	0.000	69	0.862	0.138	51	1.000	0.000
Chulinak	1988	100	1.000	0.000	99	0.823	0.177	99	0.879	0.121	99	1.000	0.000	99	0.960	0.040	99	0.950	0.051	0.000	99	0.960	0.035	0.005	0.000	99	0.939	0.061	99	1.000	0.000
Anvik	1987	150	1.000	0.000	150	0.827	0.173	150	0.883	0.117	150	1.000	0.000	49	0.987	0.013	150	0.980	0.020	0.000	150	0.927	0.067	0.007	0.000	115	0.913	0.087	106	1.000	0.000
	1988	100	1.000	0.000	100	0.740	0.260	100	0.870	0.130	100	1.000	0.000	98	0.985	0.015	92	0.935	0.065	0.000	100	0.935	0.045	0.020	0.000	99	0.773	0.227	100	0.995	0.005
Rodo	1989	77	1.000	0.000	77	0.792	0.208	77	0.883	0.117	77	1.000	0.000	77	0.981	0.020	77	0.955	0.046	0.000	77	0.909	0.058	0.026	0.007	77	0.903	0.097	77	1.000	0.000
Nulato																														
Main stem	1987	61	1.000	0.000	61	0.787	0.213	61	0.853	0.148	61	1.000	0.000	61	0.951	0.049	61	0.918	0.082	0.000	61	0.902	0.090	0.008	0.000	58	0.922	0.078	61	1.000	0.000
South Fork	1987	71	1.000	0.000	71	0.754	0.247	71	0.901	0.099	71	1.000	0.000	50	0.990	0.010	71	0.951	0.049	0.000	71	0.930	0.056	0.014	0.000	69	0.862	0.138	71	1.000	0.000
North Fork	1988	50	1.000	0.000	50	0.830	0.170	50	0.920	0.180	50	1.000	0.000	50	0.980	0.020	50	0.980	0.020	0.000	50	0.880	0.090	0.030	0.000	50	0.930	0.070	50	0.990	0.010
	Pooled	182	1.000	0.000	182	0.786	0.214	182	0.863	0.137	182	1.000	0.000	61	0.972	0.028	182	0.948	0.052	0.000	182	0.907	0.077	0.017	0.000	177	0.901	0.099	182	0.997	0.003
Glasaa	1989	97	1.000	0.000	97	0.809	0.191	97	0.918	0.083	97	1.000	0.000	97	0.985	0.016	97	0.974	0.026	0.000	97	0.912	0.083	0.005	0.000	97	0.897	0.103	97	1.000	0.000
Koyukuk																														
Henshaw	1987	43	1.000	0.000	43	0.919	0.081	43	0.895	0.105	43	1.000	0.000	43	0.977	0.023	43	0.942	0.058	0.000	43	0.919	0.070	0.012	0.000	43	0.907	0.093	43	1.000	0.000
Jim	1987	101	1.000	0.000	101	0.921	0.079	101	0.916	0.084	101	1.000	0.000	101	0.965	0.035	101	0.951	0.050	0.000	101	0.876	0.124	0.000	0.000	100	0.915	0.085	91	1.000	0.000
So. Fork, Early	1990	74	1.000	0.000	74	0.919	0.081	74	0.885	0.115	74	1.000	0.000	74	1.000	0.000	74	0.973	0.027	0.000	74	0.885	0.108	0.007	0.000	74	0.858	0.142	74	1.000	0.000
So. Fork, Late	1990	75	1.000	0.000	75	0.907	0.093	75	0.947	0.053	75	1.000	0.000	75	0.973	0.027	75	0.953	0.047	0.000	75	0.847	0.133	0.020	0.000	75	0.900	0.100	75	1.000	0.000
	Pooled	293	1.000	0.000	293	0.916	0.084	293	0.913	0.087	293	1.000	0.000	93	0.978	0.022	293	0.956	0.044	0.000	293	0.877	0.114	0.009	0.000	292	0.896	0.104	283	1.000	0.000
Tozitna	1989	85	1.000	0.000	85	0.871	0.129	85	0.900	0.100	85	1.000	0.000	85	0.982	0.018	85	0.941	0.059	0.000	85	0.847	0.141	0.012	0.000	85	0.859	0.141	85	1.000	0.000
Salcha	1988	48	1.000	0.000	48	0.927	0.073	48	0.896	0.104	50	1.000	0.000	50	0.990	0.010	48	0.958	0.042	0.000	48	0.896	0.094	0.010	0.000	48	0.885	0.115	50	1.000	0.000
	1989	50	1.000	0.000	50	0.850	0.150	50	0.930	0.070	50	1.000	0.000	50	0.980	0.020	50	0.940	0.060	0.000	50	0.910	0.060	0.030	0.000	50	0.840	0.160	50	1.000	0.000
	Pooled	100	1.000	0.000	98	0.888	0.112	98	0.913	0.087	100	1.000	0.000	100	0.985	0.015	98	0.949	0.051	0.000	98	0.903	0.077	0.020	0.000	98	0.862	0.138	100	1.000	0.000
Yukon River (Fall)																														
Tolstat	1987	135	1.000	0.000	135	0.878	0.122	135	0.937	0.063	135	1.000	0.000	128	0.992	0.008	135	0.959	0.041	0.000	136	0.789	0.182	0.030	0.000	86	0.919	0.081	85	1.000	0.000
	1990	74	1.000	0.000	73	0.932	0.068	74	0.939	0.061	74	1.000	0.000	74	0.987	0.014	74	0.987	0.014	0.000	74	0.757	0.216	0.027	0.000	73	0.904	0.096	74	1.000	0.000
	Pooled	209	1.000	0.000	208	0.897	0.103	209	0.938	0.062	209	1.000	0.000	200	0.990	0.010	209	0.969	0.031	0.000	209	0.778	0.194	0.029	0.000	159	0.912	0.088	159	1.000	0.000
Delta	1987	135	1.000	0.000	135	0.907	0.093	132	0.943	0.057	135	1.000	0.000	134	0.963	0.037	136	0.978	0.014	0.000	135	0.796	0.182	0.022	0.000	85	0.915	0.085	135	1.000	0.000
	1990	75	1.000	0.000	75	0.873	0.127	75	0.933	0.067	75	1.000	0.000	75	0.973	0.027	75	0.960	0.040	0.000	75	0.820	0.167	0.013	0.000	75	0.913	0.087	75	1.000	0.000
Bluff Cabin	1987	135	1.000	0.000	135	0.915	0.085	135	0.933	0.067	135	1.000	0.000	123	0.947	0.053	135	0.956	0.044	0.000	135	0.852	0.130	0.019	0.000	75	0.920	0.080	135	1.000	0.000
	Pooled	345	1.000	0.000	345	0.903	0.097	342	0.937	0.063	345	1.000	0.000	332	0.959	0.041	345	0.965	0.035	0.000	345	0.823	0.158	0.019	0.000	215	0.916	0.084	345	1.000	0.000
Chandalar	1987	149	1.000	0.000	135	0.915	0.085	135	0.933	0.067	149	1.000	0.000	123	0.947	0.053	135	0.956	0.044	0.000	149	0.785	0.191	0.024	0.000	33	0.879	0.121	150	1.000	0.000
	1988	73	1.000	0.000	73	0.925	0.075	73	0.938	0.062	73	1.000	0.000	73	0.993	0.007	73	0.980	0.020	0.000	73	0.781	0.212	0.007	0.000	73	0.829	0.171	72	1.000	0.000
	1989	75	1.000	0.000	75	0.893	0.107	75	0.887	0.113	75	1.000	0.000	75	1.000	0.000	75	0.973	0.027	0.000	80	0.756	0.231	0.013	0.000	80	0.863	0.138	80	1.000	0.000
	Pooled	297	1.000	0.000	297	0.909	0.091	297	0.921	0.079	297	1.000	0.000	294	0.983	0.017	294	0.975	0.025	0.000	297	0.793	0.192	0.015	0.000	181	0.865	0.135	297	1.000	0.000
Sheenjek	1987	135	1.000	0.000	135	0.911	0.089	135	0.896	0.104	135	1.000	0.000	124	0.980	0.020	135	0.974	0.026	0.000	135	0.830	0.163	0.007	0.000	87	0.793	0.207	87	1.000	0.000
	1988	79	1.000	0.000	79	0.956	0.044	79	0.892	0.108	80	1.000	0.000	80	0.969	0.031	79	0.968	0.032	0.000	79	0.810	0.177	0.013	0.000	80	0.863	0.138	80	1.000	0.000
	1989	80	1.000	0.000	80	0.956	0.044	80	0.856	0.144	80	1.000	0.000	80	0.994	0.006	80	0.969	0.031	0.000	80	0.756	0.231	0.013	0.000	80	0.863	0.138	80	1.000	0.000
	Pooled	295	1.000	0.000	294	0.935	0.065	294	0.884	0.116	295	1.000	0.000	284	0.981	0.019	294	0.971	0.029	0.000	294	0.804	0.185	0.010	0.000	245	0.837	0.163	247	1.000	0.000
Fishing Branch	1987	129	1.000	0.000	128	0.934	0.066	126	0.913	0.087	129	1.000	0.000	23	0.935	0.065	129	0.973	0.027	0.000	129	0.845	0.151	0.004	0.000	71	0.937	0.063	127	1.000	0.000
	1988	49	1.000	0.000	49	0.939	0.061	49	0.898	0.102	49	1.000	0.000	49	0.980	0.020	49	0.980	0.020	0.000	49	0.827	0.174	0.000	0.000	49	0.867	0.133	49	1.000	0.000
	Pooled	178	1.000	0.000	177	0.935	0.065	175	0.909	0.091	177	1.000	0.000	72	0.965	0.035	178	0.975	0.025	0.000	178	0.840	0.157	0.003	0.000	120	0.908	0.092	176	1.000	0.000
Main Stem																														
Big Creek	1987	69	1.000	0.000	69	0.877	0.123	69	0.949	0.051	69	1.000	0.000	69	0.957	0.044	69	0.949	0.051	0.000	69	0.797	0.203	0.000	0.000	45	0.967	0.033	63	1.000	0.000
Tatchun	1987	75	1.000	0.000	75	0.900	0.100	75	0.913	0.087	75	1.000	0.000	75	0.967	0.033	75	0.967	0.033	0.000	74	0.811	0.182	0.007	0.000	66	0.886	0.114	66	1.000	0.000
Minto	1989	100	1.000	0.000	100	0.930	0.070	100	0.950	0.050	100	1.000	0.000	100	0.970	0.030	100	0.965	0.035	0.000	100	0.860	0.135	0.005	0.000	100	0.870	0.130	100	1.000	0.000
	Pooled	244	1.000	0.000	244	0.906	0.094	244	0.939	0.061	244	1.000	0.000	244	0.965	0.035	244	0.981	0.019	0.000	243	0.827	0.169	0.004	0.000	211	0.896	0.104	229	1.000	0.000
Kluane	1987	150	1.000	0.000	135	0.985	0.015	142	0.947	0.053	135	1.000	0.000	125	0.992	0.008	135	1.000	0.000	0.000	143	0.888	0.112	0.000	0.000	91	0.868	0.132	144	1.000	0.000
Teslin	1989	95	1.000	0.000	95	0.916	0.084	95	0.968	0.032	95	1.000	0.000	95	0.816	0.184	95	1.000	0.000	0.000	95	0.853	0.147	0.000	0.000	95	0.790	0.211	95	1.000	0.000
Bristol Bay Area																														
Kuskokwim																														
Kwethlok	1989	77	0.994	0.006	77	0.779	0.221	77	0.851	0.149	77	1.000	0.000	77	0.935	0.065	77	0.968	0.026	0.006	77	0.948	0.052	0.000	0.000	77	0.922	0.078	75	1.000	0.000
Aniak	1989	75	0.980	0.020	75	0.747	0.253	75	0.820	0.180	75	1.000	0.000	75	0.960	0.040	75	0.973	0.027	0.000	75	0.920	0.060	0.020	0.000	75	0.927	0.073	75	1.000	0.000
	Pooled	152	0.987	0.013	152	0.763	0.237	152	0.836	0.164	152	1.000	0.000	152	0.947	0.053	152	0.970	0.026	0.003	152	0.934	0.056	0.010	0.000	152	0.924	0.076	152	1.000	0.000
Kanektok	1989	75	1.000	0.000	75	0.753	0.247	75	0.813	0.187	75	1.000	0.000	75	0.987	0.013	75	0.960	0.040	0.000	75	0.933	0.067	0.000	0.000	75	0.893	0.107	75	1.000	0.000
Goodnews	1988	50	0.980	0.010	50	0.820	0.380	50	0.920	0.080	50	1.000	0.000	50	0.970	0.030	50	0.940	0.060	0.000	50	0.930	0.050	0.020	0.000	50	0.880	0.120	50	1.000	0.070
	1989	50	1.000	0.000	50	0.650	0.350	50	0.910	0.090	50	1.000	0.000	50	0.950	0.050	50	0.980	0.020	0.000	50	0.900	0.080	0.020	0.000	50	0.930	0.070	50	1.000	0.000
	Pooled	100	0.995	0.005	100	0.635	0.365	100	0.915	0.085	100	1.000	0.000	100	0.960	0.040	100	0.980	0.040	0.000	100	0.915	0.085	0.020	0.000	100	0.905	0.095	100	1.000	0.000
Togiak	1987	98	1.000	0.000	98	0.899	0.301	90	0.867	0.133	24	1.000	0.000	98	0.959	0.041	98	0.969	0.031	0.000	98	0.939	0.051	0.010	0.000	98	0.898	0.102	98	1.000	0.000
Nushagak	1987/88	87	1.000	0.000	87	0.741	0.259	87	0.879	0.121	87	1.000	0.000	87	0.971	0.029	87	0.977	0.023	0.000	87	0.920	0.057	0.023	0.000	87	0.805	0.195	87	1.000	0.000
Alagnak	1987	98	1.000	0.000	98	0.755	0.245	98	0.939	0.061	30	0.983	0.017	98	0.980	0.020	98	0.974	0.026	0.000	98	0.918	0.071	0.010	0.000	98	0.903	0.097	100	1.000	0.000
Naknek	1988	73	1.000	0.000	74	0.743	0.257	74	0.858	0.142	74	1.000	0.000	73	1.000	0.000	74	0.966	0.034	0.000	74	0.946	0.054	0.000	0.000	74	0.865	0.135	74	1.000	0.000
Gertrude	1987	100	1.000	0.000	100	0.785	0.215	100	0.935	0.065	32	1.000	0.000	100	0.985	0.015	100	0.965	0.035	0.000	100	0.940	0.055	0.005	0.000	100	0.860	0.140	100	1.000	0.000
Mother Goose	1987	100	1.000	0.000	100	0.805	0.195	99	0.934	0.066	100	1.000	0.000	100	0.975	0.025	100	0.970	0.030	0.000	100	0.880	0.115	0.005	0.000	58	0.828	0.172	100	1.000	0.000
Meshik	1989	75	1.000	0.000	75	0.907	0.093	75	0.900	0.100	75	1.000	0.000	75	1.000	0.000	75	0.973	0.027	0.000	75	0.913	0.087	0.000	0.000	75	0.913	0.087	75	1.000	0.000
Herendeen	1987	100	1.000	0.000	100	0.865	0.135	97	0.809	0.191	100	1.000	0.000	100	1.000	0.000	100	0.980	0.020	0.000	100	0.765	0.210	0.025	0.000	98	0.902	0.098	100	1.000	0.000
Nelson/Sapsuck	1987	100	1.000	0.000	100	0.825	0.175	100	0.970	0.030	25	1.000	0.000	100	1.000	0.000	100	1.000	0.000	0.000	100	0.915	0.080	0.005	0.000	56	0.946	0.054	100	1.000	0.000
Russia																														
Magadan	1991	78	1.000	0.000	80	0.856	0.144	80	0.925	0.075	77	1.000	0.000	79	1.000	0.000	79	0.911	0.089	0.000	80	0.850	0.125	0.025	0.000	80	0.975	0.025	80	1.000	0.000
Anadyr	1991	80	1.000	0.000	80	0.762	0.237	80	0.919	0.081	80	1.000	0.000	80	1.000	0.000	80	0.975	0.025	0.000	79	0.886	0.114	0.000	0.000	80	0.919	0.081	80	1.000	0.000
Kanchalan	1991	79	1.000	0.000	78	0.776	0.224	79	0.899	0.101	78	1.000	0.000	49	1.000	0.000	78	0.961	0.019	0.000	78	0.897	0.071	0.032	0.000	46	0.957	0.033	79	1.000	0.000

Determining Continent of Origin of Chum Salmon (*Oncorhynchus keta*) Using Genetic Stock Identification Techniques: Status of Allozyme Baseline in Asia

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A three-agency program was initiated in 1989 to develop a new multilocus genetic baseline for chum salmon (*Oncorhynchus keta*) in Japan and Russia for use in stock identification; allele frequencies at 77 allozyme loci are reported in 38 samples covering most of its north–south limits of distribution in Asia. In a 62-locus data set for 17 Japanese and 12 Russian samples, average heterozygosity ranged from 0.066 to 0.087 (mean 0.079) and the average number of $P_{0.95}$ and $P_{0.99}$ loci was 14 and 26, respectively. Tests of year-to-year variation in allele frequencies were not significant at five of six locations. For the $P_{0.95}$ loci, F_{ST} values ranged from 0.007 (*sMDH-B1**) to 0.154 (*mAAT-2**) and averaged 0.038. A clear distinction between Russian and Japanese samples was observed at Nei's $D = 0.006$, and genetic differentiation generally followed a regional pattern within each country. Principal component analysis of the $P_{0.95}$ loci revealed a large difference between Japanese and Russian samples. Four loci (*sAAT-1,2**, *mAAT-2**, *LDH-A1**, and *PEPLT**) had high loadings on the first two principal components. Analyses of a simulated fishery with 200 fish revealed a high degree of precision in estimating contributions to seven population groups and to country of origin.

Un programme relevant de trois organismes a été entreprise en 1989 en vue de créer une nouvelle base génétique multilocus pour le saumon kéta (*Oncorhynchus keta*) au Japon et en Russie à des fins d'identification des stocks; on présente ici les fréquences alléliques de 77 loci d'allozymes de 38 échantillons couvrant la plupart des limites nord–sud de la distribution en Asie. Dans une série de données de 62 loci portant sur 17 échantillons du Japon et 12 échantillons de la Russie, l'hétérozygotie moyenne variait de 0,006 à 0,087 (moyenne de 0,079) et le nombre moyen de loci $P_{0.95}$ et $P_{0.99}$ était de 14 et de 26, respectivement. Les tests sur la variation de la fréquence allélique d'une année à l'autre n'étaient pas significatifs dans cinq des six endroits. En ce qui concerne les loci $P_{0.95}$, les valeurs de F_{ST} se situaient entre 0,007 (*sMDH-B1**) et 0,154 (*mAAT-2**) et leur moyenne était de 0,038. Une distinction nette entre les échantillons de la Russie et du Japon a été observée à $D = 0,006$ (Nei) et la différenciation génétique correspondait généralement à un profil régional pour chaque pays. L'analyse en composantes principales des loci $P_{0.95}$ a révélé une différence importante entre les échantillons du Japon et ceux de la Russie. Quatre loci (*sAAT-1,2**, *mAAT-2**, *LDH-A1**, et *PEPLT**) ont montré des charges élevées dans les deux premières composantes principales. Des analyses d'une pêche simulée de 200 poissons ont révélé un haut degré de précision dans l'estimation des contributions à sept groupes de population et au pays d'origine.

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Chum salmon (*Oncorhynchus keta*) is the most widely distributed species of salmon in the North Pacific Ocean. It is found in steams and rivers of various sizes around the Pacific Rim from Monterey, California, in North America to Korea and southern Honshu in Asia (Neave et al. 1976). It is the second most abundant species of salmon and, historically, may have constituted 50% of the biomass of Pacific salmon in the North Pacific Ocean (Salo 1991). The annual commercial harvest of chum salmon in the North Pacific Ocean from 1925 to 1981 ranged from 18 to 83 million fish, averaging 41 million fish (Salo 1991).

Chum salmon is one of the most important commercial species of salmon in Japan and Russia. In Japan, the species is managed entirely in a hatchery system that accounts for over 90% of the salmon produced nationally by aquaculture (Kaeriyama 1989). There are approximately 300 hatcheries in Japan that release about 2 billion fry annually (Kaeriyama and Urawa 1992). Between 40 and 60 million adult chum salmon return to the Japanese coasts every year (Kaeriyama and Urawa 1992). In Far East Russia, the chum salmon fishery is based primarily on native populations. The average annual catch of chum salmon in Russia from 1981 to 1992

was 16.4 million adults (N.V. Varnavskaya, unpublished data). There are 10 chum salmon hatcheries on Sakhalin Island that release approximately 150–200 million fry per year (Kanidiev and Juikova 1970).

For the latter half of this century, high seas Japanese fisheries for salmon, including chun salmon, generated interest in determining the continent of origin of these fish. Under the aegis of the International North Pacific Fisheries Commission, authorized under the International Commission for the High Seas Fisheries of the North Pacific Ocean between Japan, Canada, and the United States in 1952, a coordinated international effort to study stock origins of Pacific salmon in high-seas fisheries focused on tagging experiments and scale pattern analysis (see Davis et al. 1990). Regional studies of stock identification of chum salmon have used characters such as gonad development, scale characteristics, age, morphology, and in some instances, protein genetic (alloyzme) characters (e.g., Altukhov et al. 1980; Okazaki 1986; Nikolaeva 1987; Ishida et al. 1989).

Interest in determining the origin of salmon caught in the North Pacific Ocean increased in the 1980s because of their incidental catch in a high-seas driftnet fishery for flying squid (*Ommastrephes bartrami*). Begun in the late 1970s with the deline of a nearshore jigging fishery, the high-seas drifnet fishery increased in size until, by the late 1980s, it included approximately 750 ships from Japan, Taiwan, and Korea, and had an average annual harvest of 200 000 – 300 000 t of squid (Yatsu 1990). Because of the invasive nature of the driftnet technique, the incidental take or bycatch of other pelagic species, including salmon, became an international concern to the fisheries community. Because of their quantitative prominence in the high-seas pelagic community, chum salmon have been an important bycatch in driftnets.

Sufficient levels of allozyme variation have been described among populations in every species of Pacific salmon to allow the use of genetic stock identification (GSI) methodology (Milner et al. 1985) in some aspect of fisheries management (e.g., Grant et al. 1980, sockeye salmon (*Oncorhynchus nerka*); Shaklee et al. 1991, pink salmon (*O. gorbuscha*); Milner 1993, coho salmon (*O. kisutch*); Utter et al. 1987b, chinook salmon (*O. tshawytscha*). Allozyme variation in chum salmon has been described by Okazaki (1982), Salmenkova et al. (1986), and Beacham et al. (1985, 1987). Winans et al. (1990, 1991) demonstrated the feasibility of estimating the continent of origin of chum salmon populations in hypothetical mixed-stock fisheries using a preliminary data set (5 loci and 93 populations). Although earlier genetic studies in Asia covered extensive geographic portions of Japan and Far East Russia (Okazaki 1982; Salmenkova et al. 1986, 1992; Altukov and Salmenkova 1990; Olmelchenko et al. 1992), few loci were investigated. With the development and collection of data for new polymorphic loci throughout North America (Kondzela et al. 1994; Phelps et al. 1994; Wilmot et al. 1994), the weakest segment in a species-wide base line for chum salmon was in Japan and Russia. Thus, in 1989, a cooperative research program between the Northwest Fisheries Science Center, National Marine Fisheries Service; the Hokkaido Salmon Hatchery, Fisheries Agency of Japan; and the Pacific Research Institute of Fisheries and Oceanography, Kamchatka, Russia, was initiated to develop a more extensive genetic base line for chum salmon in Asia.

TABLE 1. Sample sites of adult chum salmon from Russia and Japan. Location numbers refer to sites in Fig. 1.

	Location	Collection date		No of fish
	Russia			
1	Anadyr River		1991	104
2	Korf Bay	5 Aug.	1991	18
3	Ossora River	27 July	1990	40
4	Nerpichi Lake	19 July	1991	40
5A	Kamchatka River	23 Aug.	1990	80
5B	Kamchatka River	2 Aug.	1991	40
6	Adrianovka River	4 Sept.	1991	36
7A	Avacha River	1 July	1990	32
7B	Avacha Bay	23 Aug.	1991	26
8	Utka River		1991	79
9	Kikchik River		1991	40
10A	Pymta River	29 July	1990	80
10B	Pymta River		1991	79
11	Kol River	30 July	1990	93
12	Vorovskaia River		1991	40
13	Hairusova River	3 July	1990	154
14	Gizhiginskaya Bay		1989	80
15	Tumani River	15 July	1991	66
16	Tauy River	26 July	1990	57
17A	Ola River	3 Aug.	1990	80
17B	Ola River	15 July	1991	80
	Japan			
18	Masuhoro River	19 Oct.	1987	80
19	Teshio River	15 Oct.	1987	97
20A	Chitose River	16 Oct.	1989	100
20B	Chitose River	16 Oct.	1990	80
21	Yurrapu River	8 Oct.	1989	100
22	Gakko River	8 Nov.	1989	39
23	Miomote River	6 Nov.	1989	100
24	Tokushibetsu River	17 Oct.	1987	42
25	Tokoro River	11 Nov.	1988	100
26	Shari River	18 Oct.	1989	100
27	Shibetsu River	9 Oct.	1988	100
28	Nishibetsu River	17 Oct.	1989	100
29	Kushiro River	19 Oct.	1989	100
30A	Tokachi River	20 Oct.	1989	100
30B	Tokachi River	27 Sept.	1990	80
31	Tsugaruishi River	7 Dec.	1989	100
32	Ohkawa River	6 Dec.	1989	100

This paper describes the level of allozyme variability within populations in Japan and Russia and the pattern of genetic differentiation among populations, and evaluates the new multilocus data base in analyses of computer-simulated mixed-stock fisheries. When standardized with new data from North American stocks of chum salmon (Kondzela et al 1994; Phelps et al 1994; Wilmot et al. 1994; L. Seeb, Alaska Dept. Fish and Game, Anchorage, AK, personal communication), these collective data will form the most comprehensive GSI base line for any species of Pacific salmon for use in fisheries research, management, and forensics.

Materials and Methods

Tissues were collected from adult fish at weirs in natal streams (Japan) and in stream surveys (Russia) from 1987 to 1991 (Table 1, Fig. 1) with the exception of fish sampled in a nearshore area of Gizhiginskaya Bay by gillnets (see

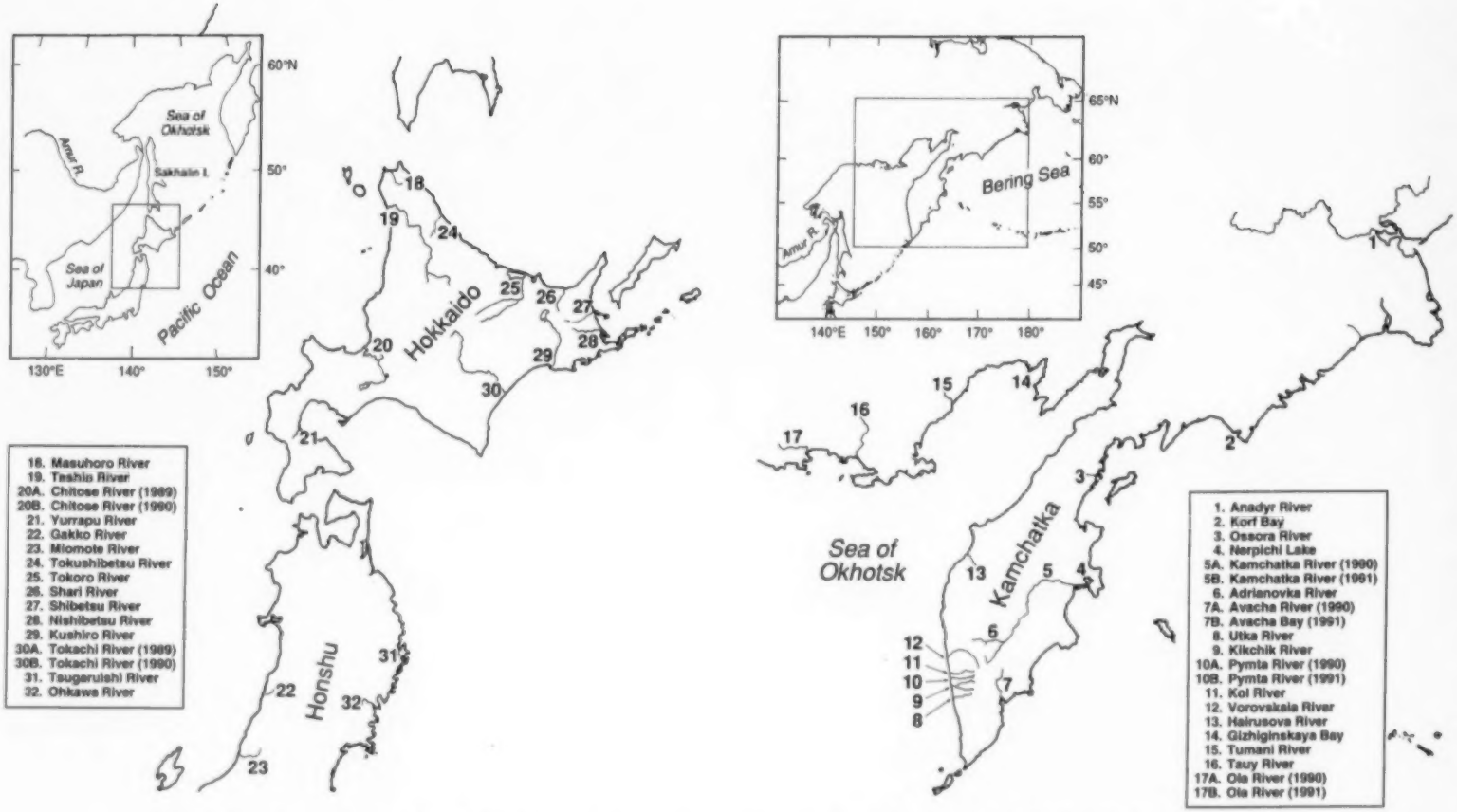

FIG. 1. Locations in Japan (left) and Russia where chum salmon samples were taken from 1987 to 1991. Sample dates are given in Table 1.

TABLE 2. Enzymes and electrophoretic conditions to resolve 77 loci in samples of Asian chum salmon. Enzyme commission (EC) numbers follow IUBMBNC (1992). Tissues are muscle (M), heart (H), liver (L), and retinal tissue from the eye (E). The TC4 gel buffer is described in Harris and Hopkinson (1976), where the pH is adjusted with HCl; all others are described in Aebersold et al. (1987). The ACN6 is a modified AC6 with the addition of 15 mg NAD^+ (β-nicotinamide adenine dinucleotide) per 100 mL of the starch solution and 30 mg NAD^+ in the cathodal electrode buffer. Variation at iosloci was assigned to one locus (see text).

Enzyme name	EC No.	Locus abbreviation	Tissue	Gel buffer	Samples with data[a]	Samples polymorphic	Level of variation[b]
Alanine aminotransferase	2.6.1.1	*mAAT-1**	L, H	ACE7	38	30	≤0.95
		*mAAT-2**	L, H	ACE7	37	34	≤0.95
		*sAAT-1,2**	M, H	TBE	38	38	≤0.95, M
		*sAAT-3**	E	TBCLE	35	35	≤0.95
Acid phosphatase	3.1.3.2	*ACP**	L	TBE	37N	3	≤0.99
Adenosine deaminase	3.5.4.4	*ADA-2**	M, E, H	TBE	29N	0	M
Aconitate hydratase	4.2.1.3	*mAH-1,2**	H	ACE7	35N	7	≤0.99, M
		*mAH-3**	H	ACE7	38	38	≤0.95
		*mAH-4**	H	ACE7	38	2	≤0.99
		*sAH**	L	ACE7	37	14	≤0.99
Adenylate kinase	2.7.4.3	*AK**	M	ACE7	33N	0	M
Alanine aminotransferase	2.6.1.2	*ALAT**	M	TBE	36	36	≤0.95
Creatine kinase	2.7.3.2	*CK-A1**	M	TBCLE	36	4	≤0.99
		*CK-A2**	M	TBCLE	35	4	≤0.99
		*CK-B**	E	TBCLE	35	0	M
		*CK-C1**	E	TBCLE	31	3	>0.99
		*CK-C2**	E	TBCLE	27N	12	≤0.99
Esterase-D	3.1.–.–	*ESTD**	M	TBCLE	38	38	≤0.95
Formaldehyde dehydrogenase (glutathione)	1.2.1.1	*FDHG**	L	TBE	33	2	≤0.99
Fumarate hydratase	4.2.1.2	*FH**	M	ACE7	38	27	≤0.95
β-*N*-Acetylgalactosaminidase	3.2.1.53	*βGALA**	L	ACE7	35	35	≤0.95
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	M	ACE7	37	0	M
		*GAPDH-2**	H	ACN6	37	11	≤0.95
		*GAPDH-3**	H, M	ACN6	38	4	≤0.95
		*GAPDH-4**	E	ACE7	34	0	M
		*GAPDH-5**	E	ACE7	32N	4	≤0.99
Glycerol-3-phosphate dehydrogenase (NAD^+)	1.1.1.8	*G3PDH-1**	M	ACE7	37	1	>0.99
		*G3PDH-2**	H	ACN6	37	35	≤0.95
		*G3PDH-3**	H	ACN6	37	4	≤0.99
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A**	M	TBCLE	38	5	≤0.99
		*GPI-B1,2**	M	TBCLE	37	10	>0.99, M
Glutathione reductase	1.6.4.2	*GR**	E	TBCLE	36	0	M
β-Glucuronidase	3.2.1.31	*βGUS**	L	TBCL	38	0	M
β-*N*-Acetylhexosaminidase	3.2.1.52	*βHEX**[c]	L	TC4	38	35	≤0.95
L-Iditol-2-dehydrogenase	1.1.1.14	*IDDH-1**	L	TBCL	35N	1	>0.99
		*IDDH-2**	L	TBCL	35N	12	≤0.95
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	*mIDHP-1**	M	ACE7	38	38	≤0.95
		*sIDHP-1**	L	ACE7	38	6	≤0.95
		*sIDHP-2**	L	ACE7	38	38	≤0.95
L-Lactate dehydrogenase	1.1.1.27	*LDH-A1**	M	ACE7	37	37	≤0.95
		*LDH-A2**	M	ACE7	37	0	M
		*LDH-B1**	M, E	TBCLE	38	5	≤0.99
		*LDH-B2**	L, M, E	TBCLE	37	17	≤0.95
		*LDH-C**	E	TBCLE	36	2	>0.99
Lactoylglutathione lyase	4.4.1.5	*LGL**	M	TBCLE	34N	1	<0.99
α-Mannosidase	3.2.1.24	*αMAN**	L, E, H	TBE	37N	0	M
Malate dehydrogenase	1.1.1.37	*mMDH-1**	M, H	ACE7	34N	0	M
		*mMDH-2**	M, H	ACE7	37	1	>0.99
		*sMDH-A1**	L, E, H	ACE7	33N	24	≤0.95
		*sMDH-A2**	L, E, H	ACE7	38	12	≤0.99
		*sMDH-B1,2**	M, E, H	ACE7	38	33	≤0.95, M

TABLE 2. (*Continued*)

Enzyme name	EC No.	Locus abbreviation	Tissue	Gel buffer	Samples with data[a]	Samples polymorphic	Level of variation[b]
Malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$)	1.1.1.40	*mMEP-2**	M	ACE7	37	37	≤0.95
		*sMEP-1**	M	ACE7	36	16	≤0.95
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	L, H	TBE	38	38	≤0.95
Nucleoside-triphosphate pyrophosphatase	3.6.1.19	*NTP**	M	TBCLE	35N	17	≤0.95
Dipeptidase	3.4.–.–	*PEPA**	M, E	TBE	37	10	<0.95
Tripeptidase	3.4.11.4	*PEPB-1**	L, M, E	TBE, TC4	38	38	≤0.95
Proline dipeptidase	3.4.13.9	*PEPD-1**	M, H	TBE	38	7	≤0.99
Leucyl-L-tyrosine peptidase	3.4.–.–	*PEPLT**	M	TBCLE	33	28	≤0.95
Phosphogluconate dehydrogenase (decarboxylating)	1.1.1.44	*PGDH**	L, M, E	ACE7	38	34	≤0.95
Phosphoglycerate kinase	2.7.2.3	*PGK-1**	M, E, H	ACE7	38	1	>0.99
		*PGK-2**	M, E, H	ACE7	36	0	M
Phosphoglucomutase	5.4.2.2	*PGM-1**	M, E, H	ACE7	38	6	≤0.95
		*PGM-1r**	M, E, H	ACE7	38	34	≤0.95
		*PGM-2**	M, E, H	ACE7	38	7	≤0.99
Pyruvate kinase	2.7.1.40	*PK-1**	H	ACE7	38	0	M
		*PK-2**	H	ACE7	38	1	>0.99
Superoxide dismutase	1.15.1.1	*sSOD-1**	L	TBE	38	1	>0.99[d]
Triose-phosphate isomerase	5.3.1.1	*TPI-1**	E, H	TBE	38	2	<0.99[d]
		*TPI-2**	E, H	TBE	38	1	>0.99[d]
		*TPI-3**	E, H	TBE	38	5	≤0.99
		*TPI-4**	E, H	TBE	38	8	≤0.99
Xanthine oxidase	1.1.3.22	*XO**	L	TBCL	32N	0	M

[a]N = locus that was not part of the "complete" data set.
[b]Level of variation: M = all samples monomorphic; in one or more samples, if the frequency of the common allele was greater than 0.99, then ">0.99", if it was greater than 0.95 and less than or equal to 0.99, then "≤0.99", and if it was less than or equal to 0.95, then "≤0.95".
[c]Formerly referred to as EC 3.2.1.30 (βGLUA).
[d]This locus is monomorphic in the "complete" data set.

Winans et al. 1990). Samples of muscle, heart, liver, and retinal tissue were frozen on dry ice or liquid nitrogen and transported to −80°C freezers (or held on liquid nitrogen) until shipping to and processing at the Northwest Fisheries Science Center in Seattle, Wash.

Samples were examined for protein electrophoretic variation on horizontal starch gels using standard procedures described by Aebersold et al. (1987). Interpretation of the banding patterns followed the methods described by Utter et al. (1987a). Laboratory conditions for each enzyme are presented in Table 2. Standard nomenclature for loci and alleles was used as outlined in Shaklee et al. (1990a). In general, multiple loci for an enzyme were designated with hyphenated numerals, with the locus encoding for the least anodal homomer (identical protein subunits) designated as 1, the next as 2, and so on. Alleles were referred to numerically, with the most common allele at a locus designated 100. Other alleles were assigned numbers representing the mobility of their homomeric protein relative to the migration distance of the homomeric protein of allele 100.

Allozyme data were collected for up to 77 loci. Because of tissue degradation, complete data for 62 loci were collected from only 12 of the 21 Russian samples, however all available data for the Russian samples are included for future reference. We refer to a 62-locus – 29-sample data set as the "complete" data set. Only the 62-locus data set was used to estimate levels of genetic variation. We also report the laboratory conditions for an additional 15 loci not resolved in all samples (Table 2) because of the potential use of these data in a species-wide data set.

Genetic analyses were conducted with the computer program BIOSYS (Swofford and Selander 1981). The amount of genetic variation in a sample was estimated as (1) H, the average heterozygosity per locus (calculated from the heterozygous proportions expected under Hardy–Weinberg equilibrium), (2) the average number of alleles per locus, and (3) the proportion of polymorphic loci per sample. A locus was considered polymorphic if more than one allele was detected, and was considered a $P_{0.95}$ locus or $P_{0.99}$ locus if the frequency of the common allele was ≤0.95 or ≤0.99, respectively. Estimates of genetic variation in Russian and Japanese samples were compared in t-tests.

To evaluate the magnitude of population differentiation, two single-locus measures, F_{ST} (Wright 1978) and ΔP, were calculated for the $P_{0.95}$ loci. F_{ST} ranges from 0, indicating no population differentiation, to a value of 1, indicating fixation for alternate alleles. ΔP is defined as the absolute value of the difference between the average allele frequency for the 100 allele in Russia and in Japan. Year-to-year variation in allele frequencies at a locale was evaluated by calculating pairwise G-tests between years for each polymorphic locus and summing G-values. For a multilocus estimate of population differentiation, Nei's (1978) unbiased genetic distance statistic was calculated for all pairwise comparisons of populations, and genetic relationships among populations were depicted in a dendrogram by clustering the genetic

TABLE 3. Convention used to recode genotypes to numeric scores for principal component analysis. In general, for a locus with x alleles, $x - 1$ new variables (referred to as Y_i) are generated. Actual genotypes at *sIDHP-2** are recoded in the example of a four-allele locus where the "pool" class represents the **25*, **20*, and **110* alleles.

	Two-allele locus									
	100/100	50/100	50/50							
Y_1 =	1.0	0.50	0.00							
	Three-allele locus									
	100/100	50/100	30/100	50/50	30/50	30/30				
Y_2 =	1.0	0.5	0.5	0.0	0.0	0.0				
Y_3 =	0.0	0.5	0.0	1.0	0.5	0.0				
	Four-allele locus									
	100/100	35/100	85/100	pool/100	35/35	35/85	35/pool	85/85	85/pool	pool/pool
Y_4 =	1.0	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Y_5 =	0.0	0.5	0.0	0.0	1.0	0.5	0.5	0.0	0.0	0.0
Y_6 =	0.0	0.0	0.5	0.0	0.0	0.5	0.0	1.0	0.5	0.0

distance estimates with a UPGMA algorithm (Sneath and Sokal 1963).

A principal component (PC) analysis was conducted using StatSoft (1991) on a correlation matrix constructed from coded genotypic data for the $P_{0.95}$ loci. In this method, genotypes were converted to scores of (1, 0.5, 0) as described by Smouse and Neel (1977) and Smouse et al. (1982); a two-allele locus yielded one new coded variable; a three-allele locus yielded two coded variables, and so on (Table 3). For statistical simplicity, infrequently occurring alleles (i.e., frequency less than 0.01) were pooled with other allele classes of similar mobility.

To evaluate the data in a standard genetic stock identification application, we analyzed a series of computer-simulated mixtures using the EM algorithm (Millar 1987). We used the 25 $P_{0.95}$ loci, and phenotypic frequencies at *PGM-1r**; simulated mixtures consisted of 200 fish with equal contributions from 29 samples. Estimates were made to individual populations; after 100 repetitions, the results were summarized as mean estimates for population groups that were identified in genetic distance and principal component analyses. Actual proportions were set for each population as 100%/29 = 3.45% and then pooled to reflect the number of populations in each group. The base line was resampled and convergence criterion was set at 1.2×10^{-5} (Millar 1987).

Results

Allele frequencies for 77 loci are presented in the Appendix. Whereas 14 of the 62 loci (23%) were monomorphic, most loci (60%) had two or three alleles per locus. Eight loci had four alleles per locus, *PEPB-1** had five alleles, and *sIDHp-2** and *sMDH-B1,2** had six alleles each.

Three isoloci (i.e., where products of two gene loci are indistinguishable or comigrate on a gel) were variable: *GPI-B1,2**, *sMDH-B1,2**, and *sAAT-1,2**. Allelic variation at *GPI-B1,2** was rare: alleles **145* and **40* were observed in only 10 samples at a frequency of ≤0.012. Allelic variation at *sMDH-B1,2** reached the $P_{0.95}$ level: **95* was found in approximately 75% of the samples at a frequency up to 0.056, and **65* was found in 15 of the 17 Japanese samples at a maximum frequency of 0.068 and in two of the 12 Russian samples at a frequency of ≤0.012. Three other alleles (*115, *120, and *85) were rarely seen. Allelic variation at *sAAT-1,2** reached the $P_{0.95}$ level: **120* was observed in all samples and reached a frequency of 0.31 (Tumani River). The **65* and **113* alleles were rare in samples from Gizhiginskaya Bay, Tauy River, Masuhoro River, and Tokushibetsu River.

Although the variation at the isoloci could not unambiguously be assigned to one or the other of the locus pairs (e.g., Shaklee and Phelps 1992), we assigned the variation at each isolocus pair to one locus for two reasons. First, no triple-dose heterozygotes, which can only result from variation at two polymorphic loci, were observed (see Utter et al. 1987a). Second, the variation did not differ significantly ($P < 0.05$) from Hardy–Weinberg equilibrium when tested as single loci. Waples (1988) showed that mistakenly assuming that a single locus is responsible for all the variation at an isolocus will, on average, lead to an apparent deficiency of heterozygotes. Finally, this treatment of isoloci further simplifies the principal component analysis and the variability estimates.

A null allele was detected at *PGM-1** in addition to a mobility variant (**−110*). Because of the inability to distinguish the two heterozygous phenotypes involving the null allele (**null* – **−100* and **null* – **−110*), phenotypic frequencies of the null homozygote were recorded as a separate locus *PGM-1r**. This follows a standardized procedure for coastwide management of allozyme data for chum salmon (S. Phelps, Washington Department of Fisheries, Olympia, WA, personal communication). Variation at *PGM-1r** was used in the genetic distance calculations and principal component analysis, but excluded from the Hardy–Weinberg tests and calculation of average heterozygosity per locus. The frequency of the null phenotype averaged about 0.04 in most samples, with a maximum frequency of 0.11 at Ohkawa River in Japan and 0.22 at Nerpichi Lake in Russia.

Of the 48 polymorphic loci in the "complete" data set, eight had rare variation (i.e., where the frequency of secondary alleles was <0.010) and 14 were polymorphic at the $P_{0.99}$ level (Table 2). Twenty-six loci were polymorphic at the $P_{0.95}$ level (Table 4). Five of the $P_{0.95}$ loci reached this level in only one or two samples (*GAPDH-2**, *GAPDH-3**, *sMDH-B1,2**, *sMEP-1**, and *PGM-1**), whereas

six loci reached the $P_{0.95}$ level in all 29 samples (*sAAT-3**, *mAH-3**, *ALAT**, *EST-D**, *sIDHP-2**, and *mMEP-2**). The distribution of variability was substantially different at six loci. For *sAAT-1,2**, *mAAT-2**, *G3PDH-2**, and *MPI**, 10–12 Russian samples were variable at the $P_{0.95}$ level, whereas only three or four Japanese samples were variable at this level. And, whereas 100 and 59% of the Japanese samples reached the $P_{0.95}$ level for *HEX** and *PEPLT**, only 50 and 8% of the Russian samples reached the $P_{0.95}$ level, respectively (Table 4).

Within-Sample Variation

In the complete data set, 631 comparisons of observed genotypic frequencies were made against Hardy–Weinberg expectations; 44 of the tests were statistically significant at the $P < 0.05$ level. The number of significant tests per sample ranged from zero in five samples to three in five samples (Utka River, Shari River, Chitose River 1989 and 1990, and Tokushibetsu River). The overall proportion of departures from Hardy–Weinberg expectations (7.0%) is slightly larger than the proportion expected by chance alone for 631 comparisons (95% confidence interval: 3.3–6.7%). In many cases, the significant tests involved expected genotypic frequencies of less than 1 (e.g., four of the six significant tests for *mIDHP-1** involved expected frequencies of <1 for **60/*60*). In these cases, the test statistic may not follow the chi-square distribution (Sokal and Rohlf 1981). We concluded that there is no evidence for substantial deviation from Hardy–Weinberg equilibrium and we assumed that the samples consisted of individuals from single panmictic populations.

Estimates of the level of variability in each sample with data for 62 loci are summarized in Table 5. Average heterozygosity ranged from 0.066 in Kushiro River 1989 to 0.087 in Tumani River and averaged 0.079 over 29 samples (SD = 0.004). The mean H value for the 17 Japanese chum salmon samples ($H = 0.077$) was statistically different from the 12 Russian samples ($H = 0.081$; $t = 2.166$, df = 27, $P = 0.039$). The percentage of $P_{0.99}$ loci per sample ranged from 27.9 to 41.0%, and averaged 34.4% overall. There was no significant difference between the Japanese (35.1%) and Russian samples (33.3%) for this estimate. The percentage of $P_{0.95}$ loci ranged from 18.0 to 27.9%, with a mean of 23.7%. Japanese samples had fewer $P_{0.95}$ loci than Russian samples ($t = 2.84$, df = 27, $P = 0.008$). The average number of alleles per locus ranged from 1.34 in Korf Bay to 1.66 in Miomote River, with a mean of 1.50 (SD = 0.069). This estimate did not differ significantly between the Japanese and Russian samples.

Reliable data were collected at an additional 15 loci in some of the samples (Appendix). Five loci were polymorphic at the $P_{0.95}$ level: *mAH-1,2**, *CK-C2**, *IDDH-2**, *sMDH-A1**, and *NTP**, with the latter two loci exhibiting the most variation. Twelve of the 13 samples scored in Japan for *sMDH-A1** were polymorphic at the $P_{0.95}$ level; the **200* allele reached a frequency of 0.21 in Teshio River. Variation at *sMDH-A1** in Russia reached only the $P_{0.99}$ level in 10 of 20 samples. About half the samples were variable for *NTP**, four of which reached the $P_{0.95}$ level in Russia. The **65* allele reached a maximum frequency of 0.22 in Korf Bay. *CK-C2** was polymorphic only in the Japanese samples. Four loci were polymorphic at the $P_{0.99}$ level (*ACP**, *GAPDH-5**, *LGL**, and *IDDH-1**) and five loci (*ADA-2**, *AK**, *αMAN**, *mMDH-1**, and *XO**) plus a presumed locus at *mAH-1,2** were monomorphic.

TABLE 4. Estimates of the magnitude of population differentiation in 26 $P_{0.95}$ loci for 29 samples of chum salmon. ΔP = difference in average frequency of the common allele (**100*) between Russia and Japan.

Locus	No. of $P_{0.95}$ samples[a]	F_{ST}	ΔP
*sAAT-1,2**	13 (10,3)	0.018	0.052
*sAAT-3**	29 (12,17)	0.022	0.096
*mAH3**	29 (12,17)	0.028	0.057
*mAAT-1**	6 (3,3)	0.038	0.024
*mAAT-2**	15 (12,3)	0.154	0.262
*ALAT**	29 (12,17)	0.016	0.060
*EST-D**	29 (12,17)	0.051	0.153
*FH**	7 (2,5)	0.023	0.030
*HEX**	23 (6,17)	0.067	0.149
*bGALA**	23 (12,11)	0.021	0.084
*G3PDH-2**	15 (12,3)	0.037	0.093
*GAPDH-2**	1 (0,1)	0.018	0.016
*GAPDH-3**	1 (1,0)	0.030	0.008
*mIDH-1**	23 (12,11)	0.080	0.047
*sIDH-1**	1 (1,0)	0.024	0.008
*sIDH-2**	29 (12,17)	0.015	0.055
*LDH-A1**	27 (11,16)	0.017	0.021
*PEPB-1**	27 (10,17)	0.079	0.168
*PEPLT**	16 (1,15)	0.057	0.090
*sMDH-B1,2**	1 (0,1)	0.007	0.010
*sMEP-1**	2 (0,2)	0.031	0.025
*mMEP-2**	29 (12,17)	0.038	0.114
*MPI**	16 (12,4)	0.021	0.071
*PGDH**	7 (5,2)	0.021	0.038
*PGM-1**	2 (0,2)	0.047	0.007
*PGM-1r**	13 (6,7)	na[b]	0.013

[a]The numbers in parentheses are the number of Russian and Japanese samples, respectively.
[b]na = not available.

Temporal Variation

Temporal variation was examined at the 62 loci (when available) at Tokachi River, Chitose River, Ola River, Kamchatka River, Pymta River, and Avacha River–Bay. An overall statistically significant difference was seen between the 1989 and 1990 samples from Chitose River (sum-G = 57; df = 41, $P = 0.04$). In this comparison, significant differences were seen at *mAH-3** ($P = 0.014$) and *ALAT** ($P = 0.025$). Significant differences between years at individual loci were seen for *sAAT-1,2** in Tokachi River ($P < 0.005$) and for *MPI** in Ola River ($P = 0.024$). Because five of the six populations did not vary significantly over 2 yr, we conclude that year-to-year variability in allele frequencies is not substantial in this data set.

Among-Population Variation

Two single-locus estimates of the magnitude of population differentiation for the $P_{0.95}$ loci are given in Table 4. F_{ST} values ranged from 0.007 (*sMDH-B1**) to 0.154 (*mAAT-2**) and averaged 0.038 over the 25 $P_{0.95}$ loci. Seven loci had F_{ST} values greater than 0.05. Values from 0.05 to 0.15 indicate moderate levels of differentiation (Hartl 1980). Most of the ΔP values were between 0.04 and 0.10. Five loci had

TABLE 5. Summary of variability estimates in 29 samples of chum salmon for 62 allozyme loci.

	Location	H^a	% of loci $P_{0.95}$	% of loci $P_{0.99}$	Alleles per locus
	Russia				
1	Anadyr River	0.081	22.95	29.50	1.45
2	Korf Bay	0.084	22.95	31.15	1.34
4	Nerpichi Lake	0.082	26.23	34.43	1.45
5A	Kamchatka River	0.076	24.59	36.06	1.50
5B	Kamchatka River	0.071	24.59	31.15	1.36
8	Utka River	0.077	24.59	27.87	1.43
9	Kikchik River	0.084	24.59	36.06	1.45
10B	Pymta River	0.079	26.23	34.43	1.52
11	Kol River	0.081	27.87	37.71	1.63
15	Tumani River	0.087	26.23	37.71	1.52
17A	Ola River	0.086	24.59	34.43	1.52
17B	Ola River	0.081	24.59	29.50	1.59
	Japan				
18	Masuhoro River	0.079	22.95	32.78	1.56
19	Teshio River	0.075	21.31	34.43	1.49
20A	Chitose River	0.076	21.31	40.98	1.54
20B	Chitose River	0.079	19.67	31.15	1.59
21	Yurrapu River	0.080	22.95	34.43	1.56
22	Gakko River	0.076	19.67	37.71	1.46
23	Miomote River	0.083	22.95	39.34	1.66
24	Tokushibetsu River	0.082	22.95	37.71	1.49
25	Tokoro River	0.081	21.31	37.71	1.54
26	Shari River	0.077	22.95	31.15	1.46
27	Shibetsu River	0.076	24.59	31.15	1.50
28	Nishibetsu River	0.077	22.95	37.71	1.52
29	Kushiro River	0.066	18.04	32.78	1.46
30A	Tokachi River	0.076	24.59	36.06	1.50
30B	Tokachi River	0.077	27.87	32.78	1.50
31	Tsugaruishi River	0.074	24.59	32.78	1.50
32	Ohkawa River	0.081	26.23	36.06	1.46

[a]Average heterozygosity calculated for 61 loci; *PGM-1r** was excluded (see text).

ΔP values greater than 0.10; the largest ΔP was 0.262 for *mAAT-2**. Average ΔP was 0.070.

Multilocus between-population variation was estimated by Nei's genetic distance (D) values. A 29-sample dendrogram of genetic distances based on 62 loci is illustrated in Fig. 2. The largest separation of samples was observed between the Russian and Japanese groups at $D = 0.006$. Within the Russian samples, the Anadyr River and Utka River samples formed branch VII at $D = 0.0047$; the two Kamchatka River samples were also clearly differentiated at $D = 0.0025$ (branch VI). Of the remaining eight Russian samples, branch IV contained three west Kamchatka Peninsula samples (Kikchik River, Pymta River, and Kol River) and the Ola River 1991 (the "Magadan" area); branch V contained the Tumani River (from northern Sea of Okhotsk), Ola River 1990, and two Bering Sea samples (Nerpichi Lake and Korf Bay).

Of the Japanese samples, eight samples from seven locales in northeast and east Hokkaido (sample numbers 24–30) grouped together (branch I) and were distinctive from other Japanese samples at $D = 0.002$. Branch II contained the remaining samples from Hokkaido (three samples along the Sea of Japan and Yurrapu River on the southern peninsula) and three of the four samples from Honshu. The Ohkawa River sample (branch III) was distinctive from branch II at $D = 0.0014$. With the exception of those from the Miomote River, the most southerly sample site on Honshu, samples along the Sea of Japan (Masuhoro River, Teshio River, Chitose River, and Gakko River) clustered together at $D = 0.0006$ within branch II.

The 21 Russian samples were compared using a dendrogram of genetic values when the number of loci was reduced to 35 (listed in the legend of Fig. 3). The pattern of differentiation was similar to the pattern in the 62-locus analysis. For example, six of the seven samples from west Kamchatka Peninsula clustered together on branch R1. The samples from the Magadan and northern Sea of Okhotsk areas (Ola River 1990 and 1991, Tumany River, and Gizhiginskaya Bay) and the Bering Sea area (Korf Bay, Ossora River, and Nerpichi River) grouped together in branch R2. The Tauy River sample (from the Magadan area) was similar to the west Kamchatka Peninsula samples on branch R1; the two Avacha River samples, from southeast Kamchatka Peninsula, were similar to Magadan – Bering Sea samples in branch R2. Adrianovka River, from the Kamchatka River drainage, grouped with the other Kamchatka River samples (branch R3).

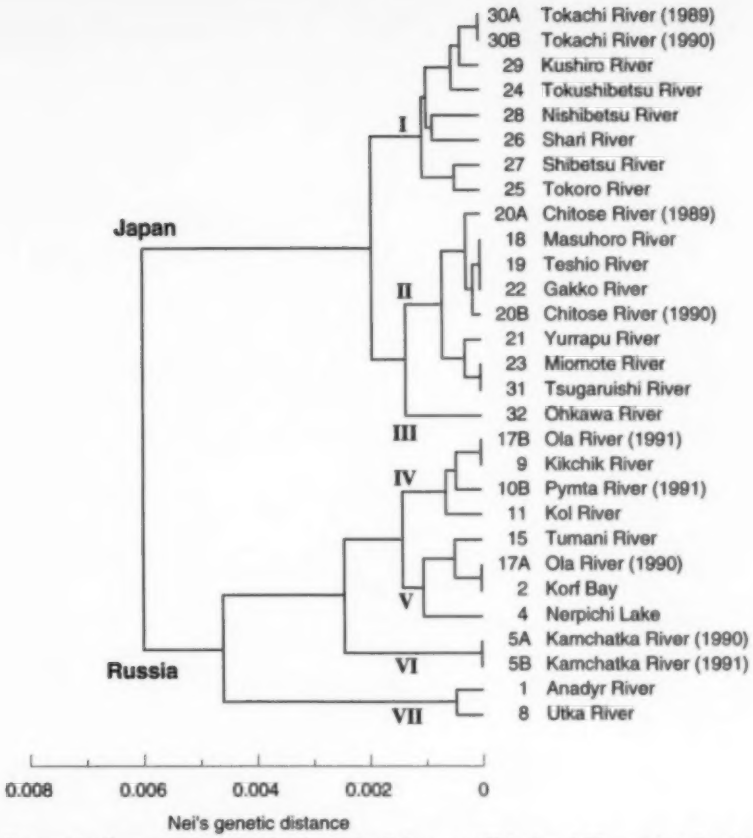

FIG. 2. Dendrogram of Nei's genetic distance values for the "complete" data set (62 loci and 29 samples) for chum salmon from Russia and Japan.

Results of the principal (PC) analysis are summarized in Table 6 and Fig. 4. Principal component analysis requires complete data for any fish included in the analysis. To maximize the number of fish in the analysis, three loci (*mAH-3**, *sAAT-3**, and *sIDHP-1**) were excluded because of the level of incomplete data: 17, 14, and 4% of the total fish had missing data at each of these loci, respectively. The first three eigenvectors explain 21% of the total variation (Table 6). Although this was considerably less variation than is commonly seen along the first vectors (Utter et al. 1989; G.A. Winans, unpublished data), valuable among-population differences are revealed along these multilocus axes (as defined by the eigenvectors). The remaining components explain incrementally less variation along axes that are dominated by variation at single loci each and therefore, are not discussed here.

Four loci had high factor loadings on PC1 and PC2. On PC1, large positive loadings for *sAAT-1,2*100, mAAT-2*100,* and *PEPLT*85* contrasted with negative loadings for *sAAT-1,2*120* and *PEPLT*100.* PC2 was a contrast of positive loadings for *sAAT-1,2*100* and *LDH-A1*100* and negative loadings for *sAAT-1,2*120* and *LDH-A1*130.* Analyses of variance of factor scores of PC1 and PC2 indicated statistically significant differences between Japan and Russia ($P < 0.001$). PC3 had high loadings for *ALAT*100, ALAT*93,* and *sIDHP-2*35*; however, a comparison of PC3 scores between Japan and Russia was not significant ($P = 0.68$).

A scatterplot of mean factor scores per sample on PC1–PC2 axes illustrates the difference between Japanese and Russian samples (Fig. 4): Japanese samples had positive values on PC1 and Russian samples had negative values. The highest PC1 values were seen in the most southerly samples from Japan, with the exception of the Gakko River. There was little variability among the Russian samples for PC1. PC2 values were generally greater than zero in the Japanese samples (with the exception of Shari River and Yurrapu River) but varied considerably among the Russian samples. For example, Tumani River had the lowest mean PC2 value (−0.67) followed by a three-sample group from the west Kamchatka Peninsula and the two Ola River samples. Samples from east Kamchatka Peninsula had PC2 values between 0 and 0.11, and the Anadyr River and Utka River samples had PC2 values greater than 0.3.

Computer-Simulated Mixture Analysis

The results of the computer-simulated mixture analysis based on the allele frequencies at the 25 $P_{0.95}$ loci and the phenotypic frequencies at *PGM-1r** are presented in Fig. 5. Results are pooled to the seven groups, I–VII, as identified in Fig. 2. Estimates were generally close to the actual values and the precision ranged from 2.4 to 5.4% (SD). When the estimates are pooled to national origin, the estimates are indistinguishable from the actual values: 57.7% (SD = 4.5) (estimate) versus 58% (actual) for Japan and 42.3%

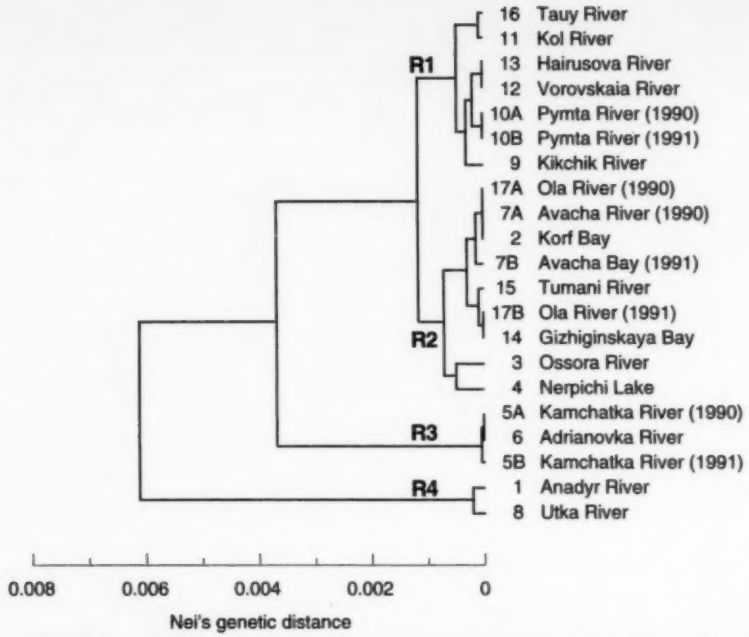

FIG. 3. Dendrogram of 21 samples of chum salmon from Russia. Nei's genetic distance values are based on the following 35 loci: *mAAT-1*, sAAT-1,2*, mAH-1,2*, mAH-3*, mAH-4*, ESTD*, FH*, βGLUA*, GAPDH-3*, G3PDH-3*, GPI-A*, βGUS*, mIDHP-1*, sIDHP-1*, sIDHP-2*, LDH-B1*, LDH-B2*, αMAN*, sMDH-A2*, sMDH-B1,2*, MPI*, PEPB-1*, PGDH*, PGK-1*, PGM-1*, PGM-1r*, PGM-2*, PEPD-1*, PK-1*, PK-2*, sSOD-1*, TPI-1*, TPI-2*, TPI-3**, and *TPI-4**.

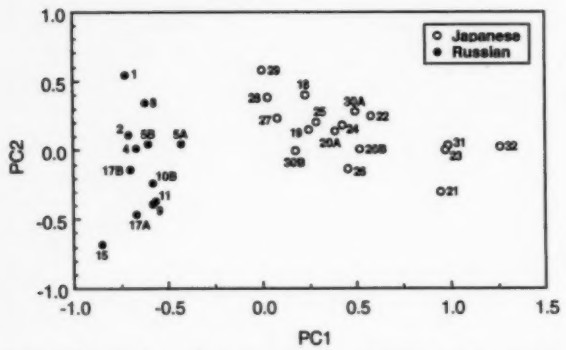

FIG. 4. Scatterplots of scores on PC1 and PC2 for 29 samples of chum salmon from Russia and Japan. Locale numbers are provided in Table 1 and Fig. 1.

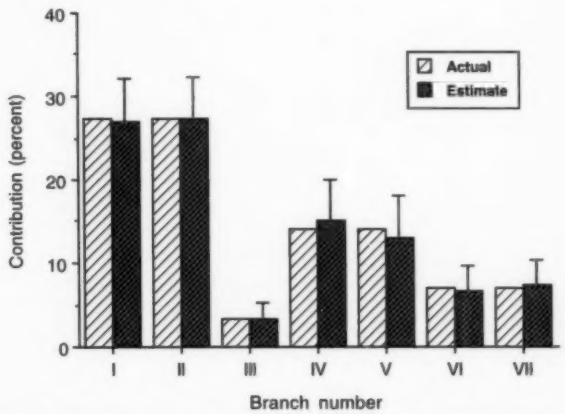

FIG. 5. Results of simulated mixture analysis. Estimates of contributions (SD) are pooled to population "groups" identified by branch nos. I–VII in Fig. 2.

(SD = 4.5) (estimate) versus 42% (actual) for Russia. Although this result is not surprising, given that population estimates were pooled with genetically similar populations (Pella and Milner 1987), it clearly demonstrates the potential reliability of these data for GSI applications at the level of country of origin.

Discussion

Although earlier allozyme studies of chum salmon populations in Russia and Japan covered a wide range of populations, they were limited in the number of loci surveyed. Salmenkova et al. (1986) studied chum salmon from the Anadyr River southward to the southern tip of Sakhalin Island, but examined only a maximum of 13 loci. They collected some data for the polymorphic loci *mIDHP-1**, *sIDHP-2**, *mMEP-2**, and *ESTD**, but had complete data for only *sAAT-1,2**, *LDH-A1**, *sMDH-B1,2**, *PGDH**, and *LDH-A1**. Similarly, Altukhov et al. (1980) studied variation at five loci in seven locations from the Amur River, Sakhalin Island, and the southern Kuril Islands. In Japan, a geographically extensive study of 43 samples was based on a maximum of 13 loci (Okazaki 1982). The most variable loci for which he had complete data were *sIDHP-2**, *LDH-A1**, and *PEPB-1**. Here, we describe variation at these 13 loci, as well as variation at an additional set of loci, and bring the

total number of $P_{0.95}$ loci to 26 in Asian chum salmon populations. The genetic information in this data set is comparable in geographic coverage and number of loci with multilocus surveys of allozyme variation in North America populations of chum salmon (Kondzela et al. 1994; Phelps et al. 1994; Wilmot et al. 1994).

In comparison with other species of Pacific salmon, for which data from 40 or more loci are available, our results indicate that chum salmon populations in Asia have moderate levels of allozymic variability. The lowest levels of within-population variation have been observed in sockeye salmon. G.A. Winans (unpublished data) surveyed 55 loci in 27 samples of sockeye salmon in British Columbia, Washington, and Idaho, and observed average heterozygosities of 0.010–0.036 (mean 0.028) and 16 $P_{0.95}$ loci (29%). Higher levels of variability have been observed in steelhead (*Oncorhynchus mykiss*) and chinook salmon, which have 30–38% $P_{0.95}$ loci and where average heterozygosity has ranged from 0.06 to 0.08 and from 0.03 and 0.10, respectively (Winans 1989; Waples et al. 1993). Estimates of allozymic variability in populations of Asian chum salmon (H from 0.066 to 0.087 and 42% $P_{0.95}$ loci) are at least equal to these species and comparable with the levels of variability described in North American populations of chum salmon (Kondzela et al. 1994; Phelps et al. 1994; Wilmot et al. 1994).

There is general concern that the artificial propagation of salmon populations in hatcheries may have detrimental effects on the species (Allendorf and Ryman 1987). Ishida et al. (1993) suggest that significant decreases in mean body weight in returning adults of chum salmon to Japanese and Russian rivers may be the result of increased numbers of chum salmon from Japanese hatcheries in the North Pacific Ocean. There is a specific concern that salmon enhancement may reduce within-population variability (Kaeriyama and Urawa 1992). Given that the Japanese populations are maintained entirely by hatcheries and Russian populations are not, we compared four indices of genetic variation to examine approximate trends, recognizing that natural clines in variability might also exist. We show that only two of the four indices (H and the percentage of $P_{0.95}$ loci) are significantly different between Japan and Russia and, therefore, feel there is no strong evidence that Japanese populations have lower levels of genetic variation in comparison with natural populations in Russia. In the absence of prehatchery estimates of genetic variation at individual Japanese hatcheries, the multilocus data reported here can serve only as a base line to monitor genetic variability in these populations in the future.

Genetic differentiation of Asian chum salmon follows a regional pattern, because proximate populations are generally genetically similar. The clearest example of this is the distinction between Russia and Japan; samples in the north are differentiated from southern samples as seen in the dendrogram (Fig. 2) and the scatterplot of PC1 and PC2 (Fig. 4). Regional patterns are also observed within each country. The seven populations along the north and east coastlines of Hokkaido are genetically similar (branch I, Fig. 2) and the samples along the Sea of Japan cluster together (branch II, Fig. 2). In Russia, samples from four rivers along the west Kamchatka Peninsula were genetically similar and distinctive from those of the east Kamchatka Peninsula and Kamchatka River (Figs. 2 and 3), and the most northern sample (Anadyr River) was also the most differentiated.

TABLE 6. Factor loadings from principal component (PC) analysis of $P_{0.95}$ loci. Several $P_{0.95}$ loci were excluded because of missing data (see text).

Coded variable	PC1	PC2	PC3
mAAT-1-10*	0.11	0.09	−0.30
mAAT-1-120*	−0.10	−0.11	0.26
mAAT-2-100*	0.44	0.11	0.03
*sAAT-1,2*100*	0.44	0.63	0.25
*sAAT-1,2*120*	−0.44	−0.63	−0.25
*ALAT*100*	−0.28	0.13	0.69
*ALAT*93*	0.28	−0.13	−0.69
*ESTD*100*	−0.25	−0.14	−0.06
*FH*100*	−0.41	0.11	−0.26
*FH*75*	0.31	−0.10	0.30
*βGALA*100*	0.28	0.04	0.03
*GAPDH-2*100*	−0.18	−0.01	0.06
*GAPDH-3*100*	0.25	0.08	0.04
*HEX*100*	−0.34	−0.08	0.09
*G3PDH-2*100*	0.10	0.09	0.01
*mIDHP-1*100*	0.22	−0.17	−0.04
*sIDHP-2*100*	0.19	−0.04	−0.42
*sIDHP-2*35*	−0.03	0.04	0.45
*sIDHP-2*85*	−0.23	−0.02	−0.01
*LDH-A1*100*	−0.19	0.73	−0.30
*LDH-A1*130*	0.18	−0.73	0.30
*sMDH-B1,2*100*	−0.07	−0.02	−0.16
*sMDH-B1,2*95*	−0.01	0.03	0.16
*mMEP-2*100*	0.20	−0.02	−0.02
*sMEP-1*100*	−0.17	−0.06	−0.07
*MPI*100*	0.15	−0.02	−0.11
PEPB-1-100*	−0.28	0.02	0.06
PEPB-1-146*	−0.14	−0.03	−0.10
*PEPLT*100*	−0.67	0.20	−0.01
*PEPLT*85*	0.67	−0.20	0.01
*PGDH*100*	0.16	0.06	−0.04
PGM-1-100*	−0.16	0.06	−0.08
*PGM-1r*null*	0.07	0.06	0.02
Eigenvalue	2.75	2.13	2.07
Proportion of variance explained	0.084	0.064	0.063

There are several "outliers" to this general scheme of population differentiation. For example, the Utka River sample from west Kamchatka Peninsula is genetically similar to the Anadyr River sample. Determining whether this is a real biological relationship or an artifact of sampling is difficult. No information is available concerning population histories of the respective rivers. It is unlikely that random population bottlenecks have resulted in the high genetic similarity, as neither sample has unusually low levels of genetic variability (Table 5). A separate sample from the Anadyr River area described by Wilmot et al. (1994) was not statistically different from our sample (comparing 13 polymorphic loci, the sum-G = 27.5; df = 19, P = 0.09). This confirmed our multilocus characterization of chum salmon in that area. A similar comparison cannot be made for the Utka River. It is interesting to note that the Utka River sample deviated from Hardy–Weinberg expectations at 3 of 20 loci. That the three loci were deficient of heterozygotes indicated that the 79-fish sample might be a mix of gene pools with differing allele frequencies (Wahlund effect). Repeat sampling of these river systems, particularly the

Utka River, is warranted to further understand these genetic affinities.

The Ohkawa River sample can also be considered an outlier. Although the Honshu samples from the Tsugaruishi and Miomote Rivers (along with the Yurrapu River, southern Hokkaido) were genetically similar, the sample from the Ohkawa River was considerably different. In fact, the genetic distance between Ohkawa River and its nearest neighbor on Honshu was almost as great as the genetic distance between these samples as a group (branches II and III) and the northeast and east Hokkaido samples (branch I, Fig. 2).

Okazaki (1982) examined populations along each coast of Honshu further south than those examined here. He examined seven samples south of Miomote River on the Sea of Japan coast and five samples south of Ohkawa River on the Pacific coast. He found significant allele frequency differences at *sIDHP-2** and *LDH-A1** between northern and southern samples on both coasts in the vicinity of the Miomote River and Tsugaruishi River, respectively. Okazaki relates these north and south "regional populations" to run-timing differences that adapt outmigrant fry to differences in seawater temperatures. An assessment of this north–south break in population differentiation on Honshu awaits a current multilocus survey of more southern Honshu samples (S. Urawa, unpublished data).

Other genetic studies of Pacific salmon have reported regional differentiation. Beacham et al. (1987) surveyed 43 populations of chum salmon in British Columbia (approx. 48°30′–55°N) using seven polymorphic loci. They identified clusters of genetically similar samples defined by island groups (Queen Charlotte Islands) or portions of islands (west coast of Vancouver Island), major river systems (Fraser River and its tributaries), and along major contiguous coastlines (southern mainland coast and east coast of Vancouver Island). Differentiation of chum salmon in Washington and southeast Alaska also follows regional patterns (Kondzela et al. 1994; Phelps et al. 1994). Regional patterns of geographic variation have also commonly been seen in large-scale multilocus studies of chinook salmon (Utter et al. 1989), coho salmon (*O. kisutch*) (G. Milner, Northwest Fisheries Science Center, Seattle, WA, personal communication), and pink salmon (*O. gorbuscha*) (Shaklee et al. 1991). Assuming the majority of allozyme alleles are evolutionarily neutral (Kimura 1968), the simplest explanation for this pattern of differentiation is isolation by distance. Namely, gene flow occurs most frequently among nearest neighbors.

A possible exception to this generalized view of differentiation among salmon populations occurs in sockeye salmon. The pattern of variation over 55 loci of sockeye salmon populations in the Columbia River drainage and the southern portion of the Fraser River resembles a mosaic, i.e., nearest neighbors are not necessarily highly similar (G.A. Winans, unpublished data). One possible explanation is that the extended freshwater rearing period in most sockeye salmon populations leads to a high degree of homing and thus less gene flow between neighboring populations.

Coinciding with the overall pattern of genetic differentiation for chum salmon from the sampled areas in Asia is the pattern of adult run timing, the time of year when mature or maturing adult fish enter fresh water to spawn. On a broad scale, Russian stocks from the Anadyr River southward throughout the Kamchatka Peninsula and west to the northern Sea of Okhotsk are summer-run fish, whereas Japanese populations are fall-run fish, returning from September through December (Salo 1991). The parallel nature of these two character sets, one a set of qualitative Mendelian loci and the other a multilocus, quantitative character, corroborates our principal finding that Russian and Japanese stocks are genetically dissimilar. On a finer scale, run-timing differences on Hokkaido correspond to genetic differences (Okazaki 1982). The Sea of Japan populations return in October, whereas Pacific coast populations and Sea of Okhotsk populations return in October through November. These differences generally correspond to genetic differences (Fig. 2). It is interesting to note that both summer- and fall-run populations occur in the Amur River and that populations on the east coast of Sakhalin Island are summer run and west coast Sakhalin Island populations are fall run (Salo 1991). In light of the correspondence of genetic variation and run timing, multilocus data from Amur River and Sakhalin Island populations would represent an important addition to the Asian chum salmon base line.

A basic tenet of the GSI technique is that accuracy and precision of population or population-group estimates are a function of the level of among-group differentiation (Milner et al. 1985). As recently as 1986 (Okazaki 1986), a study of stock composition of chum salmon was conducted with an analysis of biological characters along with seven allozyme loci. In this study, biological characters were assessed because "the use of genetic data alone is not sufficient to estimate the geographic origins of salmonid fishes during their pelagic phase" (Okazaki 1986). However, we describe here almost three times the previously reported number of polymorphic loci, and it is clear from the F_{ST} and ΔP values, the dendrogram and PC plots, and the computer-simulated mixture analyses that stock composition problems within Asia may be addressed accurately and precisely using GSI methodology.

Our findings look favorable in comparison with similar statistics from a successful GSI program in Puget Sound. Since 1985, the Northwest Indian Fisheries Commission and Washington Department of Fisheries have accurately monitored American and Canadian populations of chum salmon caught in northern Puget Sound fisheries using a 21-locus – 36-population base line (Shaklee et al. 1990b; TCCHUM 1992; Phelps et al. 1994). In this base line, there are 11 $P_{0.95}$ loci, and F_{ST} values that range from 0.01 to 0.06, and average 0.02. By this criterion, approximately twice the level of population differentiation has been detected in Asian populations of chum salmon. We conclude from this simple comparison that GSI applications in Asia are likely to produce accurate population composition estimates using the genetic data described here.

The most complex GSI application is in the North Pacific Ocean where both Asian and American populations are intermixed. We are in the process of incorporating available multilocus data from North America and Asia into a species-wide base line (Kondzela et al. 1994; Phelps et al. 1994; Wilmot et al. 1994). Preliminary analyses of a species-wide data set with 5 and 12 loci indicated accurate composition estimates to country of origin (Winans et al. 1990, 1991). The addition of new polymorphic loci will certainly enhance this international application.

A concurrent study of DNA-level variability in chum salmon has also identified a polymorphism that is being evaluated for use in GSI problems (Park et al. 1993). Using polymerase chain reaction to amplify a specific segment of

mitochondrial DNA, a restriction fragment length polymorphism was detected in the ND5–ND6 region. The B type allele has an average frequency of 0.80 in eight Japanese populations and is virtually absent in 26 populations in North America. Its frequency in eight Russian populations is 0.13. Importantly, the F_{ST} between Russian samples and Japanese samples is 0.574, almost four times greater than the most discriminating allozyme locus described here (*mAAT-2**).

Presently, this DNA technology is more technically intensive and, therefore, more expensive per fish than allozyme studies (Hallerman et al. 1986; W.K. Thomas, University of Missouri, Kansas City, KS, personal communication). However, these procedures may be streamlined. A simple chromographic assay developed for humans that can detect single nucleotide differences (Nickerson et al. 1990) has been adapted to survey a mitochondrial DNA polymorphism in Pacific salmon (L. Park, Northwest Fisheries Science Center, Seattle, WA, personal communication). Not all DNA surveys are successful in identifying polymorphisms (Park et al. 1993). Still, as more discriminatory marks are described in DNA-based studies, as well as in protein research, genetic stock identification of chum salmon and other Pacific salmon will become more accurate and precise, increasing its usefulness as a tool in stock assessment and management.

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Appendix

Allele frequencies for 77 loci (including four isoloci) at 32 sample locations. See Fig. 1 for sample location names. *N* = number of fish.

Locus / Allele	Sample location																			
	1	2	3	4	5A	5B	6	7A	7B	8	9	10A	10B	11	12	13	14	15	16
sAAT-1																				
N	102	16	39	39	75	38	36	31	18	76	40	72	78	76	18	31	74	62	36
*-100	.971	1.000	.962	.974	.867	.842	.750	.919	1.000	.987	.988	.965	.962	.947	1.000	1.000	.973	.976	.985
*-120	.000	.000	.000	.000	.087	.066	.069	.016	.000	.000	.000	.000	.000	.033	.000	.000	.027	.024	.000
*-70	.029	.000	.038	.026	.107	.092	.181	.065	.000	.013	.013	.035	.038	.020	.000	.000	.000	.000	.015
sAAT-2																				
N	104	17	39	38	76	39	36	28	18	74	40	73	79	76	35	34	76	61	0
*-100	.668	.647	.756	.789	.868	.782	.792	.625	.833	.669	.712	.699	.741	.724	.814	.676	.651	.672	.000
*-145	.332	.353	.244	.211	.132	.218	.208	.375	.167	.331	.287	.301	.259	.276	.186	.324	.349	.328	.000
sAAT-1,2 *																				
N	104	18	40	40	75	39	35	32	26	79	40	76	79	90	40	115	79	66	49
*100	.890	.862	.888	.826	.914	.872	.886	.828	.904	.874	.900	.862	.904	.816	.900	.856	.754	.690	.826
*120	.110	.138	.112	.174	.086	.128	.114	.172	.096	.126	.200	.138	.196	.184	.100	.144	.240	.310	.164
*65	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000
*113	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.010
sAAT-3																				
N	97	18	39	39	67	12	34	0	22	32	39	0	74	66	31	35	0	48	51
*100	.794	.611	.782	.551	.604	.708	.706	.000	.795	.813	.705	.000	.649	.818	.823	.686	.000	.690	.716
*90	.206	.389	.192	.449	.396	.292	.294	.000	.205	.188	.295	.000	.365	.182	.177	.314	.000	.310	.284
*110	.000	.000	.026	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000
*70	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
ACP-1																				
N	104	18	40	40	76	40	36	32	18	78	0	67	79	78	30	110	79	66	57
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*210	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
ADA-2																				
N	100	15	0	40	0	40	0	0	0	39	40	0	79	77	0	0	78	65	0
*100	1.000	1.000	.000	1.000	.000	1.000	.000	.000	.000	1.000	1.000	.000	1.000	1.000	.000	.000	1.000	1.000	.000
mAH-1,2*																				
N	98	17	36	33	74	39	27	21	22	36	38	72	78	76	29	40	79	63	33
*100	.994	1.000	1.000	1.000	.974	1.000	.944	1.000	1.000	1.000	1.000	.994	1.000	.980	1.000	1.000	1.000	.992	1.000
*50	.000	.000	.000	.000	.020	.000	.056	.000	.000	.000	.000	.006	.000	.020	.000	.000	.000	.008	.000
*81	.006	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
mAH-3																				
N	90	16	36	25	72	39	27	21	21	34	38	71	78	75	34	62	76	63	36
*100	.378	.531	.403	.500	.757	.769	.796	.548	.571	.441	.645	.739	.673	.640	.647	.667	.500	.500	.632
*124	.622	.469	.597	.500	.243	.231	.204	.452	.429	.559	.355	.261	.327	.360	.353	.333	.500	.500	.368
mAH-4																				
N	103	16	36	37	76	39	28	21	21	76	40	72	39	75	32	39	79	62	34
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.987	1.000	1.000	1.000	1.000	1.000
*120	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000
*105	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000
mAH																				
N	104	18	39	40	76	40	36	32	24	78	40	75	79	78	0	96	79	66	57
*100	1.000	1.000	.987	1.000	.993	1.000	.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	.977	1.000
*90	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*106	.000	.000	.013	.000	.007	.000	.014	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.023	.000
AK																				
N	104	18	40	40	76	39	0	32	23	76	40	0	79	91	39	93	0	63	49
*100	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
ALAP																				
N	104	17	34	40	70	39	35	32	22	68	40	0	75	90	32	41	0	64	49
*100	.841	.912	.838	.850	.879	.859	.843	.859	.818	.831	.800	.000	.807	.828	.797	.902	.000	.820	.796
*93	.159	.088	.162	.150	.121	.141	.157	.141	.182	.169	.200	.000	.193	.167	.203	.098	.000	.172	.204
*105	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.008	.000
*78	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000
CK-A1																				
N	104	18	0	32	70	39	35	32	26	72	40	0	79	90	39	55	79	65	49
*100	1.000	1.000	.000	.969	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.994	1.000	1.000	1.000	1.000	1.000	1.000
*70	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*114	.000	.000	.000	.031	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000
CK-A2																				
N	104	18	0	32	70	39	35	32	0	73	40	0	79	90	35	55	80	65	49
*100	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	.994	1.000	1.000
*110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000
*80	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
CK-B																				
N	101	18	31	39	76	40	35	0	26	53	39	0	79	90	29	114	0	66	50
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
CK-C1																				
N	101	16	0	33	62	38	0	0	0	16	39	0	79	80	0	30	0	66	44
*100	1.000	1.000	.000	1.000	1.000	1.000	.000	.000	.000	1.000	1.000	.000	1.000	1.000	.000	1.000	.000	.992	1.000
*97	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*102	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.008	.000
CK-C2																				
N	82	13	0	0	0	25	0	0	0	16	40	0	76	0	0	0	0	51	37
*100	1.000	1.000	.000	.000	.000	1.000	.000	.000	.000	1.000	1.000	.000	1.000	.000	.000	.000	.000	1.000	1.000
*97	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
ESTD																				
N	103	18	40	40	75	39	35	32	26	63	40	74	79	90	38	106	78	65	48
*100	.646	.778	.850	.813	.767	.769	.729	.875	.846	.770	.925	.926	.924	.944	.961	.939	.859	.892	.927
*91	.354	.222	.150	.188	.233	.231	.271	.125	.154	.230	.075	.074	.076	.056	.039	.061	.141	.108	.073
*110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
FGBD																				
N	103	18	0	36	74	40	19	0	0	79	40	55	79	32	0	0	72	66	47
*100	1.000	1.000	.000	1.000	1.000	1.000	1.000	.000	.000	1.000	1.000	1.000	1.000	1.000	.000	.000	1.000	1.000	1.000
*125	.000	.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
FH																				
N	104	18	40	40	75	39	35	32	26	78	40	74	79	90	39	96	80	66	49
*100	.995	.972	.975	1.000	.947	.987	1.000	1.000	1.000	1.000	.988	1.000	1.000	.950	1.000	1.000	1.000	.985	.980
*75	.005	.028	.025	.000	.053	.013	.000	.000	.000	.000	.013	.000	.000	.050	.000	.000	.000	.000	.020
*125	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.015	.000
hGALA																				
N	97	18	37	40	76	39	0	30	0	74	40	72	79	75	0	105	79	66	57
*100	.778	.833	.878	.900	.872	.859	.000	.883	.000	.892	.850	.854	.861	.887	.000	.905	.829	.879	.868
*74	.222	.167	.122	.075	.128	.141	.000	.117	.000	.108	.150	.146	.139	.113	.000	.095	.152	.106	.132
*113	.000	.000	.000	.025	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.019	.015	.000
*90	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
GAPDH-1																				
N	104	18	40	40	74	39	28	32	26	72	40	0	78	90	38	68	80	65	49
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-2																				
N	104	18	40	40	75	40	0	32	23	79	40	74	77	77	39	62	77	64	40
*100	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000	1.000
*50	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000
GAPDH-3																				
N	104	18	40	40	75	40	36	32	23	78	40	74	78	77	39	62	77	65	40
*100	1.000	.972	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.938	1.000
*117	.000	.028	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000	.062	.000
GAPDH-4																				
N	101	18	33	40	75	33	0	0	6	60	39	0	79	89	19	107	0	66	52
*100	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
GAPDH-5																				
N	101	18	34	40	75	33	0	0	0	62	39	0	79	89	21	107	0	66	52
*100	.990	1.000	1.000	1.000	1.000	1.000	.000	.000	.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
*90	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*106	.010	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
HEX																				
N	103	18	39	40	75	40	36	32	25	77	40	72	77	78	38	108	79	66	57
*100	.932	.972	.949	.938	.987	1.000	.986	.969	1.000	.903	.950	.958	.942	.955	.987	1.000	.981	.939	.904
*115	.068	.028	.051	.063	.013	.000	.014	.031	.000	.097	.050	.042	.058	.045	.013	.000	.019	.061	.096
G3PDH-1																				
N	104	18	40	40	75	39	35	32	26	79	40	0	78	90	39	116	80	66	49
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	.994	1.000	1.000	1.000	1.000	1.000
*-76	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000
G3PDH-2																				
N	104	18	39	40	74	39	0	10	20	78	39	76	78	90	31	31	74	64	49
*100	.923	.833	.859	.913	.872	.910	.000	1.000	.750	.917	.897	.938	.910	.861	.935	.871	.865	.805	.816
*90	.077	.167	.141	.087	.128	.090	.000	.000	.250	.083	.103	.061	.090	.139	.065	.129	.128	.195	.184
*131	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000
G3PDH-3																				
N	104	18	40	40	75	39	31	21	22	79	40	74	78	77	33	73	78	66	40
*100	1.000	1.000	1.000	1.000	.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*80	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000

Appendix (*Continued*)

Locus / Allele	Sample Locations																	
	1	2	3	4	5A	5B	6	7A	7B	8	9	10A	10B	11	12	13	14	15	16
GPI-A																			
N	104	18	17	40	68	39	36	15	19	79	40	76	79	90	36	38	80	65	49
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.987	1.000	1.000	1.000	1.000	.981	.985	.980
*95	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.015	.010
*105	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.013	.000	.010
GPI-B1,2*																			
N	104	18	10	40	75	39	35	32	17	79	40	66	79	90	0	52	80	66	49
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.988	1.000	.000	1.000	.994	1.000	.990
*145	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.012	.000	.000	.000	.000	.000	.000
*40	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.010
GR																			
N	101	18	39	40	76	40	32	31	26	63	39	0	79	91	40	85	0	66	56
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
GUS																			
N	89	18	40	39	76	40	36	32	26	68	40	75	79	78	35	66	67	66	57
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IDDH-1																			
N	96	17	34	40	75	40	25	15	0	77	40	73	73	73	26	84	76	59	57
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*380	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
IDDH-2																			
N	97	17	34	40	75	40	25	15	0	77	40	73	73	75	26	86	77	56	57
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*125	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*10	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
mIDHP-1																			
N	104	18	40	40	75	39	36	32	26	77	40	74	79	91	38	106	80	66	49
*100	.620	.833	.900	.762	.953	.936	.897	.875	.808	.578	.875	.919	.937	.879	.921	.920	.856	.917	.929
*60	.380	.167	.100	.237	.047	.064	.103	.125	.192	.422	.125	.081	.063	.121	.079	.080	.144	.083	.071
*85	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
sIDHP-1																			
N	104	18	40	40	75	40	36	32	25	78	40	75	79	78	30	105	40	66	57
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000	1.000	1.000	.987	1.000	1.000	1.000	1.000	1.000
*70	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.013	.000	.000	.000	.000	.000
*130	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
sIDHP-2																			
N	104	18	40	40	75	40	36	32	22	77	40	75	79	78	19	106	79	66	57
*100	.438	.417	.563	.538	.467	.525	.403	.516	.500	.396	.538	.580	.563	.532	.474	.509	.646	.553	.623
*35	.433	.333	.287	.250	.447	.375	.472	.266	.341	.455	.200	.233	.241	.202	.263	.278	.215	.227	.211
*85	.101	.083	.125	.162	.080	.063	.125	.125	.136	.110	.075	.093	.082	.103	.211	.071	.070	.144	.088
*25	.019	.167	.025	.025	.007	.038	.000	.094	.023	.032	.188	.093	.114	.064	.053	.142	.070	.076	.079
*20	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*65	.010	.000	.000	.025	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.019	.000	.000	.000	.000
LDH-A1																			
N	104	18	40	40	75	39	36	32	26	79	40	0	79	90	39	106	79	66	48
*100	.971	.917	.913	.887	.852	.885	.889	.859	.846	.930	.775	.000	.861	.703	.872	.896	.880	.826	.823
*130	.029	.083	.087	.112	.147	.115	.111	.141	.154	.070	.225	.000	.139	.217	.128	.104	.120	.174	.177
*110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*0	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-A2																			
N	104	18	40	40	76	39	36	32	26	79	40	0	79	90	39	106	80	66	48
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-B1																			
N	104	18	39	40	74	40	36	30	26	79	40	74	79	91	39	121	80	66	54
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.995	1.000	1.000	1.000	1.000	1.000
*75	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*180	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.005	.000	.000	.000	.000	.000
LDH-B2																			
N	104	18	40	40	75	40	36	32	26	79	40	78	79	91	40	154	79	66	57
*100	.995	.972	.975	.975	.987	1.000	.986	.922	1.000	1.000	.962	1.000	1.000	.984	.988	.977	.994	.962	.982
*120	.005	.028	.000	.025	.013	.000	.014	.031	.000	.000	.038	.000	.000	.016	.013	.019	.006	.038	.000
*60	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*115	.000	.000	.025	.000	.000	.000	.000	.047	.000	.000	.000	.000	.000	.000	.000	.003	.000	.000	.018
LDH-C																			
N	100	18	33	40	74	40	32	25	26	62	39	0	76	89	29	119	0	59	54
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.993	1.000	1.000	1.000	.000	1.000	1.000
*95	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000
LGL																			
N	104	18	40	40	73	39	35	32	26	79	40	0	79	90	39	106	0	65	49
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.988	.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
*93	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.000	.000	.000	.000
mMDH																			
N	102	18	35	40	76	40	36	24	24	79	40	70	79	78	40	106	36	66	57
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
sMDH-1																			
N	104	18	40	39	76	39	36	32	23	78	0	74	79	77	39	75	0	60	40
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
sMDH-2																			
N	104	18	0	36	76	39	36	32	23	79	40	74	78	77	39	75	36	64	40
*100	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000	1.000	1.000	1.000	1.000
*158	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000
sMDH-A1																			
N	104	18	40	40	75	39	36	0	23	78	40	65	78	77	39	98	80	66	40
*100	.990	1.000	1.000	1.000	.973	.987	1.000	.000	.978	1.000	.988	.985	1.000	.981	1.000	.974	.938	.955	1.000
*200	.010	.000	.000	.000	.027	.013	.000	.000	.022	.000	.013	.015	.000	.019	.000	.026	.063	.045	.000
*57	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
sMDH-A2																			
N	103	18	40	40	75	40	36	32	23	79	40	78	79	77	39	154	80	66	57
*100	1.000	1.000	.988	.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*55	.000	.000	.013	.013	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000
*150	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
sMDH-B1,2*																			
N	104	18	40	40	75	39	36	32	23	79	40	74	79	91	39	153	80	66	54
*100	.988	1.000	.988	.950	.960	.962	1.000	.968	.956	.994	.962	.972	.982	.990	1.000	.996	.982	.992	.990
*95	.004	.000	.000	.050	.034	.038	.000	.032	.044	.006	.026	.014	.018	.000	.000	.004	.018	.000	.010
*65	.004	.000	.000	.000	.000	.000	.000	.000	.000	.000	.012	.000	.000	.000	.000	.000	.000	.000	.000
*115	.004	.000	.012	.000	.006	.000	.000	.000	.000	.000	.000	.014	.000	.010	.000	.000	.000	.008	.000
*120	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*85	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
mMEP-2																			
N	103	18	39	40	75	39	34	32	24	79	36	0	79	90	36	107	79	64	49
*100	.704	.667	.808	.700	.773	.859	.897	.578	.667	.823	.806	.000	.703	.739	.958	.799	.778	.828	.888
*120	.296	.333	.192	.300	.227	.141	.103	.422	.333	.177	.194	.000	.297	.261	.042	.201	.222	.172	.112
sMEP-1																			
N	104	18	0	40	76	40	35	32	26	79	40	0	79	91	38	68	77	65	49
*100	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*90	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
MPI																			
N	104	18	40	38	75	39	36	32	26	79	40	77	78	90	38	152	72	66	57
*100	.909	.917	.875	.895	.893	.885	.944	.875	.885	.892	.875	.883	.846	.883	.908	.895	.896	.886	.956
*94	.091	.083	.125	.105	.107	.115	.056	.125	.115	.108	.125	.117	.154	.117	.092	.105	.104	.114	.044
*110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
PTP																			
N	102	18	37	40	73	37	35	32	0	79	40	0	79	90	37	103	74	62	48
*100	.936	.778	1.000	1.000	.986	.959	1.000	1.000	.000	.943	.988	.000	.949	.961	.973	1.000	.986	1.000	1.000
*65	.064	.222	.000	.000	.014	.041	.000	.000	.000	.057	.013	.000	.051	.039	.027	.000	.014	.000	.000
PEPA																			
N	104	18	40	40	75	40	35	31	21	76	40	0	79	90	35	132	78	66	54
*100	1.000	1.000	1.000	1.000	.987	.988	.971	1.000	1.000	.993	1.000	.000	.994	.994	.886	.992	1.000	1.000	1.000
*90	.000	.000	.000	.000	.013	.013	.014	.000	.000	.000	.000	.000	.000	.006	.014	.008	.000	.000	.000
*113	.000	.000	.000	.000	.000	.000	.014	.000	.000	.007	.000	.000	.006	.000	.100	.000	.000	.000	.000
PEPB-1																			
N	103	18	40	40	75	39	36	32	25	76	40	75	79	90	19	141	74	66	57
*-100	.883	.806	.938	.938	.953	.987	.944	.891	.960	.888	.900	.893	.911	.950	.868	.851	.865	.886	.930
*-146	.092	.194	.038	.050	.027	.013	.028	.094	.020	.079	.162	.060	.051	.044	.053	.121	.095	.083	.070
*-126	.024	.000	.013	.013	.020	.000	.014	.016	.020	.033	.038	.047	.038	.006	.079	.007	.020	.030	.000
*-127	.000	.000	.013	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.021	.020	.000	.000
*-72	.000	.000	.000	.000	.000	.000	.014	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
PEPD-1																			
N	104	18	39	40	75	39	32	32	25	79	40	70	79	90	39	95	79	66	49
*100	.995	1.000	1.000	1.000	.987	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.987	1.000	1.000	.995	.994	1.000	.990
*110	.005	.000	.000	.000	.013	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.005	.006	.000	.010
PEPLP																			
N	103	18	0	32	75	40	0	26	0	70	40	32	79	90	0	0	77	66	57
*100	1.000	1.000	.000	1.000	1.000	1.000	.000	.962	.000	.993	.975	.969	.949	.978	.000	.000	.955	.955	.956
*85	.000	.000	.000	.000	.000	.000	.000	.038	.000	.000	.000	.031	.032	.022	.000	.000	.032	.030	.044
*70	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*115	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.025	.000	.019	.000	.000	.000	.013	.015	.000

Appendix (*Continued*)

Locus / N / Alleles	Sample Locations																			
	1	2	3	4	5A	5B	6	7A	7B	8	9	10A	10B	11	12	13	14	15	16
PGDH																				
N	104	18	40	40	75	40	35	32	26	79	40	76	79	91	39	130	80	66	56
*100	.966	.972	.913	.962	.920	.900	.886	1.000	.962	.981	.962	.947	.956	.874	.974	.985	.938	.970	.880
*88	.034	.028	.087	.025	.080	.100	.114	.000	.038	.019	.038	.053	.044	.121	.026	.015	.063	.030	.120
*106	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*95	.000	.000	.000	.013	.000	.000	.000	.000	.000	.000	.000	.000	.000	.005	.000	.000	.000	.000	.000
*84	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGM-1																				
N	104	18	40	32	71	40	36	32	26	79	38	76	79	90	40	92	78	66	49
*-100	1.000	1.000	1.000	1.000	.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*-47	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGM-2																				
N	104	18	30	32	71	40	0	0	26	79	40	74	79	76	34	39	78	66	28
*100	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-3																				
N	104	18	40	40	80	40	36	32	26	79	40	80	79	91	40	156	80	66	57
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.995	1.000	1.000	.994	.992	1.000
*-110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.005	.000	.000	.006	.008	.000
PGM-Ir																				
N	104	18	40	40	76	40	36	32	26	79	40	74	79	90	40	117	79	66	54
*-100	.981	.944	.900	.775	.987	1.000	.944	.875	1.000	.975	1.000	.959	.949	.956	.900	.897	.924	.909	.944
*NULL	.019	.056	.100	.225	.013	.000	.056	.125	.000	.025	.000	.041	.051	.044	.100	.103	.076	.091	.056
PGM-2																				
N	104	18	40	40	75	40	35	32	25	79	40	78	79	91	40	151	79	66	57
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.984	1.000	1.000	.975	.994	.994	.995	1.000	1.000	1.000	1.000	1.000
*-117	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.006	.000	.005	.000	.000	.000	.000	.000
*-83	.000	.000	.000	.000	.000	.000	.000	.016	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*-124	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.006	.000	.000	.000	.000	.000	.000
PK-1																				
N	104	18	40	40	80	40	36	32	23	79	40	74	78	77	39	76	39	64	40
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PK-2																				
N	104	18	40	40	80	40	36	32	23	79	40	74	78	77	39	76	80	65	57
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*115	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
sMDH-1																				
N	104	18	40	40	76	40	36	32	25	78	40	75	79	78	38	110	80	66	57
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*200	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000	.000
TPI-1																				
N	104	18	40	40	76	40	36	32	26	79	40	75	79	91	39	154	79	66	57
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.987	1.000	1.000	1.000	1.000	1.000	1.000	.991
*-50	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.000	.000	.000
*-175	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.009
TPI-2																				
N	104	18	40	40	76	40	36	32	26	79	40	75	79	91	40	154	79	66	57
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.997	1.000	1.000	1.000
*85	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.003	.000	.000	.000
TPI-3																				
N	104	18	40	40	76	40	36	32	26	79	40	75	79	91	39	151	78	66	57
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.984	1.000	1.000	1.000	1.000	.994	.995	.987	1.000	1.000	1.000	1.000
*95	.000	.000	.000	.000	.000	.000	.000	.016	.000	.000	.000	.000	.006	.005	.013	.000	.000	.000	.000
*80	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
TPI-4																				
N	104	18	40	40	76	40	36	32	26	79	40	76	79	91	40	151	78	66	57
*100	1.000	1.000	1.000	1.000	.993	1.000	1.000	1.000	1.000	1.000	1.000	.993	.994	1.000	1.000	1.000	.994	1.000	1.000
*102	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.006	.000	.000
*91	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000	.000
ZO																				
N	2	16	33	0	75	40	0	32	0	0	36	70	74	71	0	106	40	64	57
*100	.000	1.000	1.000	.000	1.000	1.000	.000	1.000	.000	.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000

Locus / N / Alleles	Sample Locations																			
	17A	17B	18	19	20A	20B	21	22	23	24	25	26	27	28	29	30A	30B	31	32
sAAT-1																				
N	77	68	67	73	71	79	78	37	76	38	71	76	79	68	69	58	79	77	74
*-100	.987	.993	1.000	1.000	1.000	.994	.981	.973	.993	.987	1.000	.967	.981	.949	.964	.966	.924	.981	.946
*-120	.000	.000	.000	.000	.000	.000	.006	.000	.000	.013	.000	.020	.019	.029	.007	.000	.006	.019	.034
*-70	.013	.007	.000	.000	.000	.006	.013	.027	.007	.000	.000	.013	.000	.022	.029	.034	.070	.000	.020
sAAT-2																				
N	77	72	71	72	71	80	78	38	75	37	72	76	79	70	71	58	80	77	75
*-100	.662	.639	.965	.965	.979	.994	.994	.974	.967	.986	.924	.993	.969	.950	.965	.983	1.000	1.000	1.000
*-145	.338	.361	.035	.035	.021	.006	.006	.026	.033	.014	.076	.007	.051	.050	.035	.017	.000	.000	.000
sAAT-1,2*																				
N	80	80	80	80	80	80	80	39	80	39	80	80	80	80	80	79	80	78	78
*100	.768	.744	.914	.906	.906	.918	.894	.936	.932	.910	.956	.926	.900	.974	.956	.968	.862	.948	.948
*120	.232	.256	.076	.094	.094	.082	.106	.064	.068	.064	.044	.076	.100	.026	.044	.032	.138	.052	.052
*65	.000	.000	.010	.000	.000	.000	.000	.000	.000	.026	.000	.000	.000	.000	.000	.000	.000	.000	.000
*113	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
sAAT-3																				
N	37	68	74	69	78	78	79	33	74	36	78	72	79	73	77	76	75	80	80
*100	.716	.625	.608	.603	.596	.551	.627	.561	.574	.500	.667	.569	.652	.582	.526	.590	.567	.581	.506
*90	.284	.368	.385	.397	.391	.442	.367	.439	.412	.500	.333	.451	.348	.418	.474	.410	.453	.412	.494
*110	.000	.007	.007	.000	.013	.000	.006	.000	.014	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000
*70	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
ACP-1																				
N	78	79	79	80	80	79	80	37	78	40	78	80	80	77	79	79	71	80	71
*100	1.000	1.000	1.000	1.000	.994	.981	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000
*210	.000	.000	.000	.000	.006	.019	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000
ADA-2																				
N	79	80	80	40	74	80	80	39	80	38	80	80	80	80	79	76	80	72	80
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
mAH-1,2*																				
N	62	80	0	0	75	78	80	39	73	39	0	77	78	80	80	80	79	78	79
*100	1.000	1.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	.000	.988	1.000	1.000
*50	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.012	.000	.000
*81	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
mAH-3																				
N	63	79	31	33	38	70	80	39	70	38	37	77	73	78	80	79	78	78	79
*100	.611	.551	.532	.561	.395	.571	.637	.551	.614	.526	.632	.481	.604	.551	.456	.513	.565	.641	.532
*124	.389	.449	.468	.439	.605	.429	.363	.449	.386	.474	.368	.519	.396	.449	.544	.487	.455	.359	.468
mAH-4																				
N	59	79	31	32	76	71	80	39	75	32	37	77	75	80	80	80	79	78	79
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*120	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*105	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
sAH																				
N	80	80	79	80	77	69	80	39	78	40	77	80	79	69	62	65	58	80	80
*100	1.000	1.000	.994	.969	.987	.986	1.000	1.000	.994	.975	1.000	.994	1.000	.978	.984	1.000	.991	1.000	1.000
*90	.000	.000	.000	.019	.013	.014	.000	.000	.006	.025	.000	.000	.000	.000	.000	.000	.009	.000	.000
*106	.000	.000	.006	.013	.000	.000	.000	.000	.000	.000	.000	.006	.000	.022	.016	.000	.000	.000	.000
AK																				
N	80	80	80	80	0	80	80	39	80	40	80	80	80	40	40	40	80	0	80
*100	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000
ALAT																				
N	80	77	40	39	75	80	78	39	78	38	40	80	68	71	80	78	80	80	78
*100	.788	.864	.863	.769	.827	.719	.718	.872	.816	.697	.637	.781	.757	.887	.813	.744	.756	.831	.769
*93	.213	.136	.138	.231	.173	.281	.282	.128	.186	.303	.363	.219	.243	.113	.188	.256	.244	.169	.231
*105	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*78	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
CK-A1																				
N	80	80	80	80	77	80	80	39	80	40	80	80	79	80	80	80	80	80	80
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994
*70	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.000	.000	.000	.000	.006
*114	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
CK-A2																				
N	80	80	80	80	80	80	80	39	80	40	80	80	79	80	80	80	80	80	80
*100	1.000	1.000	1.000	.988	.988	.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*110	.000	.000	.000	.013	.013	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*80	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
CK-B																				
N	75	77	78	78	78	80	79	39	78	36	71	73	80	80	80	80	80	80	80
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Appendix (*Continued*)

Locus / Allele	Sample Locations																		
	17A	17B	18	19	20A	20B	21	22	23	24	25	26	27	28	29	30A	30B	31	32
CK-C1																			
N	75	73	77	30	78	80	80	39	78	37	79	72	80	80	79	80	75	78	80
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.993	1.000	1.000	1.000	1.000	1.000	.994	1.000
*97	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000
*102	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000	.000
CK-C2																			
N	52	53	76	12	74	76	78	39	78	30	78	69	78	70	75	79	73	78	80
*100	1.000	1.000	1.000	1.000	.993	.961	.987	.987	.994	.983	1.000	1.000	.994	.921	.993	.994	1.000	.994	.994
*97	.000	.000	.000	.000	.007	.039	.013	.013	.006	.017	.000	.000	.006	.079	.007	.006	.000	.006	.006
ESTD																			
N	80	80	76	80	77	80	80	39	80	40	80	80	80	80	80	80	80	80	72
*100	.831	.875	.625	.613	.617	.600	.650	.603	.581	.725	.712	.688	.625	.719	.775	.750	.794	.637	.764
*91	.169	.125	.375	.387	.383	.400	.350	.397	.419	.275	.275	.313	.375	.281	.225	.237	.206	.363	.236
*110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.013	.000	.000	.000
FDH0																			
N	76	40	78	79	76	80	79	39	79	37	80	77	80	79	78	79	78	79	80
*100	1.000	1.000	.987	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*125	.000	.000	.013	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.000	.000	.000	.000
FH																			
N	80	80	80	80	80	80	80	39	79	40	80	79	80	80	80	80	80	80	80
*100	.988	.994	.994	1.000	.969	.994	.894	.974	.918	.938	.988	.962	.988	.975	.962	.925	.956	.881	.900
*75	.013	.006	.006	.000	.031	.006	.056	.013	.038	.063	.013	.038	.013	.025	.038	.044	.013	.031	.044
*125	.000	.000	.000	.000	.000	.000	.050	.013	.044	.000	.000	.000	.000	.000	.000	.031	.031	.087	.056
HBALA																			
N	78	80	77	63	80	80	78	39	77	37	78	79	77	76	79	76	76	80	79
*100	.782	.837	.909	.944	.938	.956	.955	.962	.935	.851	.910	.918	.942	.939	.975	.934	.921	.975	.949
*74	.192	.156	.091	.056	.063	.044	.045	.038	.065	.149	.090	.082	.058	.061	.025	.066	.079	.025	.051
*113	.013	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*90	.013	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
GAPDH-1																			
N	80	80	80	80	77	79	80	39	80	40	80	78	80	40	40	40	69	80	78
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-2																			
N	79	80	78	80	74	80	80	39	78	39	75	79	79	75	80	78	79	79	78
*100	1.000	1.000	1.000	1.000	1.000	.994	.969	1.000	.981	.974	.980	1.000	1.000	.973	1.000	.962	.987	.949	.955
*50	.000	.000	.000	.000	.000	.006	.031	.000	.019	.026	.020	.000	.000	.027	.000	.038	.013	.051	.045
GAPDH-3																			
N	79	80	40	80	74	80	80	39	79	39	40	79	39	75	40	78	79	79	77
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000	1.000	1.000
*117	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000
GAPDH-4																			
N	79	80	78	79	40	80	80	39	80	39	79	79	80	80	79	80	80	40	64
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-5																			
N	79	80	78	79	40	80	79	39	80	39	79	79	80	80	79	80	80	0	64
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.987	1.000	1.000	1.000	1.000	.981	1.000	1.000	.000	.977	
*90	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.019	.000	.000	.000	.023
*106	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
HBE																			
N	79	77	76	79	77	79	78	36	76	39	75	73	78	75	71	72	76	78	76
*100	.981	.955	.822	.766	.734	.734	.724	.694	.730	.872	.807	.918	.910	.860	.880	.840	.862	.744	.783
*15	.019	.045	.178	.234	.266	.266	.276	.306	.270	.128	.193	.082	.090	.140	.120	.160	.138	.256	.217
G3PDH-1																			
N	80	80	79	80	79	80	79	39	80	40	80	80	80	80	80	75	80	80	80
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*-76	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
G3PDH-2																			
N	78	79	40	40	74	79	80	39	78	37	40	77	77	75	80	77	79	79	75
*100	.814	.842	.925	.975	.973	.962	.988	.987	.981	.973	.975	.955	.955	.947	.981	.968	.949	1.000	.967
*90	.186	.158	.075	.025	.027	.038	.013	.013	.019	.027	.025	.045	.032	.053	.019	.032	.051	.000	.033
*131	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.000	.000
G3PDH-3																			
N	78	80	40	40	75	80	78	38	77	39	40	78	77	76	80	78	79	78	73
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.975	1.000	.994	.987	1.000	1.000	1.000	1.000
*80	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.025	.000	.006	.013	.000	.000	.000	.000
GPI-A																			
N	77	80	79	79	77	80	80	39	80	40	80	80	80	80	80	80	80	80	80
*100	.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*95	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*105	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
GPI-B1,2*																			
N	80	80	80	80	80	80	80	39	80	40	80	80	80	80	80	80	80	80	80
*100	.981	.994	.994	1.000	.988	.994	1.000	.988	.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*145	.006	.000	.006	.000	.012	.006	.000	.012	.012	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*40	.012	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
GR																			
N	79	80	37	40	79	73	77	38	76	37	39	72	76	78	77	78	29	76	79
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GSH																			
N	79	70	78	80	74	79	80	39	78	40	74	79	80	80	79	79	76	79	78
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IDDH-1																			
N	53	78	72	28	80	0	79	37	77	23	39	76	79	80	80	78	0	69	78
*100	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	.996	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000
*380	.000	.000	.000	.000	.000	.000	.000	.000	.004	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
IDDH-2																			
N	53	78	72	28	77	0	79	37	77	23	39	79	79	79	80	78	0	69	78
*100	1.000	1.000	1.000	1.000	.994	.000	.994	.986	.987	1.000	.987	.975	.949	.994	.988	.987	.000	.978	.968
*125	.000	.000	.000	.000	.006	.000	.006	.014	.000	.000	.000	.000	.000	.000	.000	.000	.000	.022	.032
*10	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.013	.025	.051	.006	.013	.013	.000	.000	.000
MIDHP-1																			
N	80	80	80	79	77	80	80	39	80	40	79	80	79	80	80	80	80	78	80
*100	.894	.881	.913	.911	.968	.956	.913	.962	.956	.762	.854	.769	.766	.731	.856	.900	.881	.981	.981
*60	.106	.119	.087	.089	.032	.044	.087	.038	.038	.237	.146	.231	.234	.269	.144	.100	.119	.019	.019
*85	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
MIDHP-2																			
N	80	80	79	80	80	80	80	38	78	40	80	80	80	80	80	80	77	40	80
*100	1.000	1.000	1.000	1.000	.938	.962	1.000	.961	.974	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*70	.000	.000	.000	.000	.000	.006	.000	.013	.019	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*130	.000	.000	.000	.000	.063	.031	.000	.026	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
mIDHP-2																			
N	80	80	79	80	77	80	80	38	78	40	76	80	80	80	79	79	77	80	80
*100	.550	.563	.525	.569	.578	.625	.663	.618	.641	.550	.459	.500	.425	.563	.519	.532	.539	.625	.606
*35	.244	.219	.380	.387	.364	.300	.319	.342	.282	.325	.412	.369	.438	.331	.361	.380	.390	.331	.331
*85	.075	.112	.032	.006	.019	.013	.006	.013	.013	.038	.074	.025	.013	.019	.006	.038	.006	.006	.013
*25	.131	.081	.063	.038	.039	.056	.013	.026	.058	.087	.054	.106	.119	.081	.108	.051	.065	.025	.000
*20	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.050
*110	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*65	.000	.019	.000	.000	.000	.006	.000	.013	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-A1																			
N	80	80	80	80	77	79	80	39	79	40	80	80	80	80	80	80	80	80	80
*100	.781	.675	.850	.850	.825	.810	.769	.846	.854	.913	.900	.719	.844	.837	.956	.894	.831	.837	.869
*130	.219	.125	.150	.138	.156	.190	.231	.154	.146	.087	.100	.281	.156	.162	.044	.106	.169	.150	.131
*110	.000	.000	.000	.013	.013	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000
*0	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-A2																			
N	80	80	80	80	77	80	80	39	80	40	80	80	80	80	80	80	80	80	80
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-B1																			
N	79	80	80	80	80	80	80	39	80	40	80	80	80	78	80	80	80	80	80
*100	1.000	1.000	1.000	1.000	.981	.994	1.000	.987	.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*75	.000	.000	.000	.000	.019	.006	.000	.013	.013	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*160	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-B2																			
N	80	80	79	80	80	80	80	39	80	40	80	80	80	80	79	80	80	80	80
*100	.988	.981	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.981
*120	.013	.019	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.019
*60	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
*115	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
LDH-C																			
N	76	70	78	80	76	79	79	39	78	35	79	75	80	72	78	80	74	80	80
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.993	1.000	1.000	1.000	1.000	1.000
*95	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000
LGG																			
N	80	73	80	76	0	78	80	39	80	40	40	80	80	80	78	60	80	0	80
*100	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000
*93	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000

Appendix (*Concluded*)

Locus	Sample Locations																			
N / Alleles	17A	17B	18	19	20A	20B	21	22	23	24	25	26	27	28	29	30A	30B	31	32	
sMAH																				
N	80	80	77	80	37	80	80	39	78	0	78	77	80	76	75	76	80	35	75	
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
sMEH-1																				
N	79	80	77	79	0	80	40	36	73	39	79	39	39	74	80	61	80	0	70	
*100	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	1.000	
sMEH-2																				
N	79	80	76	77	38	80	39	39	76	38	76	40	40	32	40	27	80	37	78	
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*150	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
sMEH-A1																				
N	80	80	0	40	77	80	0	39	80	40	0	40	0	80	80	80	80	80	80	
*100	.981	1.000	.000	.788	.896	.969	.000	.846	.813	.875	.000	.837	.000	.919	.913	.913	.925	.919	.881	
*200	.019	.000	.000	.213	.104	.031	.000	.154	.181	.125	.000	.162	.000	.081	.087	.087	.075	.081	.119	
*57	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
sMEH-A2																				
N	80	80	79	40	77	80	80	39	80	40	40	80	80	80	80	80	80	80	80	
*100	.994	.994	.994	.988	1.000	.988	.988	1.000	1.000	1.000	1.000	1.000	.994	1.000	1.000	.981	.988	1.000	1.000	
*55	.000	.006	.006	.013	.000	.013	.013	.000	.000	.000	.000	.000	.006	.000	.000	.019	.013	.000	.000	
*150	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
sMEH-B1, 2*																				
N	80	80	79	80	80	80	80	39	80	40	80	40	80	80	80	80	80	80	80	
*100	.969	.982	.970	1.000	.988	.994	.950	1.000	.976	.962	.958	.938	.950	.964	.918	.876	.912	.988	.982	
*95	.032	.018	.018	.000	.006	.006	.006	.000	.000	.012	.018	.050	.026	.018	.032	.056	.044	.000	.000	
*65	.000	.000	.012	.000	.006	.006	.044	.000	.012	.026	.006	.012	.018	.018	.050	.068	.044	.006	.018	
*115	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	
*120	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.018	.000	.006	.000	.000	.000	.000	.000	.000	
*85	.000	.000	.000	.000	.000	.000	.000	.000	.012	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
sMEP-2																				
N	80	80	80	80	76	80	80	39	80	40	80	80	80	80	80	80	80	79	80	
*100	.875	.844	.863	.887	.921	.869	.950	.897	.906	.813	.850	.906	.875	.794	.913	.913	.925	.937	.925	
*120	.125	.156	.138	.112	.079	.131	.050	.103	.094	.188	.150	.094	.125	.206	.087	.087	.075	.063	.075	
sMEP-3																				
N	80	80	80	80	75	80	80	39	80	40	80	80	80	80	80	80	80	79	80	
*100	1.000	1.000	.975	.981	.987	.994	.994	.962	.906	1.000	.988	.994	.975	.975	.981	.988	.994	.968	.913	
*90	.000	.000	.025	.019	.013	.006	.006	.038	.094	.000	.013	.006	.025	.025	.019	.013	.006	.032	.087	
MPI																				
N	80	80	79	80	79	80	80	39	80	40	80	80	80	80	78	79	80	79	80	
*100	.881	.950	.962	.962	.987	.969	.981	.910	.969	.988	.962	.981	.994	.956	.993	.924	.944	.930	.975	
*94	.119	.050	.038	.038	.013	.031	.019	.090	.031	.013	.025	.019	.006	.044	.007	.057	.050	.070	.025	
*110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.019	.006	.000	.000	
MPP																				
N	80	77	52	40	79	79	0	39	80	40	80	40	40	80	65	78	76	78	80	
*100	.994	.994	1.000	1.000	.994	.994	.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	.992	.994	1.000	1.000	1.000	
*65	.006	.006	.000	.000	.006	.006	.000	.000	.000	.000	.000	.000	.000	.006	.008	.006	.000	.000	.000	
PEPA																				
N	80	80	80	80	80	80	80	39	80	40	80	80	80	80	78	80	80	80	80	
*100	1.000	.994	1.000	1.000	1.000	1.000	.969	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*90	.000	.006	.000	.000	.000	.000	.031	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
*113	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
PEPB-1																				
N	80	79	80	80	76	80	80	39	80	39	80	80	80	80	80	80	78	79	80	
*-100	.837	.848	.613	.700	.684	.663	.669	.667	.706	.692	.775	.875	.750	.788	.844	.738	.776	.747	.613	
*-146	.087	.095	.038	.013	.039	.013	.006	.000	.025	.013	.013	.000	.013	.000	.000	.000	.006	.006	.000	
*-126	.075	.044	.344	.281	.276	.325	.313	.333	.262	.282	.200	.125	.237	.206	.156	.262	.218	.247	.387	
*-127	.000	.013	.006	.006	.000	.000	.013	.000	.006	.013	.013	.000	.000	.006	.000	.000	.000	.000	.000	
*-72	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
PEPD-1																				
N	80	80	79	80	77	80	80	39	80	40	79	78	80	80	80	79	79	78	80	
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*110	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
PEPLP																				
N	80	80	80	74	80	80	80	39	80	40	80	80	80	80	80	80	79	80	80	
*100	.994	.994	.894	.966	.900	.887	.837	.859	.769	.938	.938	.863	.950	.956	.931	.944	.930	.825	.750	
*85	.006	.006	.075	.007	.063	.069	.144	.077	.169	.038	.063	.100	.031	.025	.019	.031	.044	.175	.219	
*70	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
*115	.000	.000	.031	.027	.038	.044	.019	.044	.050	.025	.000	.038	.019	.019	.050	.025	.025	.000	.031	
PGDH																				
N	80	80	80	80	80	80	80	39	80	40	80	80	80	79	80	80	80	80	80	
*100	.944	.906	.975	.950	.956	.938	.994	.987	.969	1.000	.969	.969	.994	.994	.994	.994	.994	1.000	1.000	
*88	.056	.075	.019	.044	.044	.038	.006	.000	.025	.000	.031	.031	.006	.006	.006	.006	.006	.000	.000	
*106	.000	.013	.006	.006	.000	.025	.000	.013	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
*95	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
*84	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
PGM-1																				
N	80	80	79	67	80	80	80	39	71	23	77	80	80	80	71	80	80	80	40	
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*-47	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
PGM-2																				
N	80	80	80	80	79	80	80	39	78	40	80	80	80	80	79	80	80	80	80	
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
PGM-3																				
N	80	80	80	80	80	80	80	39	80	40	80	80	80	80	80	80	80	80	80	
*-100	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.950	.925	
*-110	.000	.000	.000	.000	.000	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.050	.075	
PGM-3r																				
N	80	80	80	80	77	80	80	39	80	40	80	79	80	80	80	80	80	80	80	
*-100	.913	.950	.988	.962	.974	.938	.988	1.000	.962	.975	.925	.949	.962	.988	.925	.950	.938	.988	.887	
*NULL	.087	.050	.013	.038	.026	.063	.013	.000	.038	.025	.075	.051	.038	.013	.075	.050	.063	.013	.112	
PGM-3																				
N	80	80	79	80	77	80	80	39	80	40	80	80	80	80	80	80	80	80	80	
*-100	.994	.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*-117	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
*-83	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
*-124	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
PK-1																				
N	80	40	32	40	38	80	80	39	79	38	37	80	80	80	80	78	80	37	78	
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
PK-2																				
N	80	80	39	40	77	80	79	39	80	38	40	79	80	80	80	79	80	80	79	
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.994	1.000	
*115	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.006	.000	
sMDH-1																				
N	79	80	79	80	77	80	80	39	80	40	80	78	80	80	80	79	78	80	80	
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*200	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
TPI-1																				
N	80	80	79	80	80	80	80	39	80	40	80	80	80	80	80	76	80	77	80	
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*-50	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
*-175	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
TPI-2																				
N	80	80	79	80	80	80	80	39	80	40	80	80	80	80	80	76	80	77	80	
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*85	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
TPI-3																				
N	80	80	79	80	38	80	80	39	80	40	80	79	80	80	80	79	80	40	80	
*100	1.000	.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*95	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
*80	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
TPI-4																				
N	80	80	79	80	38	80	80	39	79	40	80	79	80	80	80	79	80	40	73	
*100	.994	1.000	.994	.994	1.000	1.000	1.000	1.000	1.000	.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*102	.006	.000	.006	.006	.000	.000	.000	.000	.000	.025	.000	.000	.000	.000	.000	.000	.000	.000	.000	
*91	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
XO																				
N	72	80	79	79	75	36	80	39	80	39	76	79	80	80	79	79	38	40	80	
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

[a]Variation at these isoloci was assigned to the first locus. The second locus was assumed to be monomorphic for the same fish.

Biochemical Genetic Survey of Sockeye Salmon (*Oncorhynchus nerka*) in Canada

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Allozyme variation was examined in sockeye salmon (*Oncorhynchus nerka*) from 83 distinct spawning sites representing all major sockeye-producing river systems in Canada. Of 33 nonselected loci examined, only 14 were highly polymorphic ($q > 0.05$) and 10 were less polymorphic ($0 < q \leq 0.05$). No two populations were fixed for different alleles at any locus, but allele frequencies ranged from 0.01 to 0.86 at *PGM-1** and from 0.07 to 0.89 at *ALAT**, the two most variable loci. Mean heterozygosity ranged from 2.3 to 5.6% (mean 4.1%) across all sites. Hierarchical analysis was used to partition relative gene diversity among river systems (6.3%), major drainages within a river system (2.9%), nursery lakes within drainages (7.0%), spawning sites within lakes (1.0%), and individuals within spawning sites (82.8%). Extensive differentiation among nursery lakes affords excellent opportunities for genetic stock identification within river systems, but the relatively weak regional structuring limits opportunities for coast-wide stock identification. Genetic variation at highly structured loci corroborates the view that modern populations in Canada originated from sockeye that survived the late Wisconsin Glaciation in the Bering and Columbia refuges, and also suggests the existence of coastal refuges in British Columbia.

Les auteurs ont examiné la variation des allozymes chez des saumons rouges (*Oncorhynchus nerka*) prélevés dans 83 différentes frayères représentatives de tous les principaux réseaux fluviaux canadiens fréquentés par cette espèce. Des 33 loci non sélectionnés examinés, seuls 14 étaient fortement polymorphes ($q > 0{,}05$) tandis que 10 étaient moins polymorphes ($0 < q \leq 0{,}05$). Aucune population ne présentait de variabilité en ce qui concerne des allèles différents en un locus donné, bien que la fréquence allélique allait de 0,01 à 0,86 à *PGM-1**, et de 0,07 à 0,89 à *ALAT**, les deux loci les plus variables. L'hétérozygosité moyenne se situait entre 2,3 et 5,6 % (moyenne de 4,1 %) pour tous les sites. Les auteurs ont utilisé une analyse hiérarchique pour répartir la diversité relative des gènes entre les réseaux fluviaux (6,3 %), les principaux bassins versants de chaque réseau fluvial (2,9 %), les lacs d'alevinage de chaque bassin versant (7,0 %), les frayères dans chaque lac (1,0 %) et les individus dans chaque frayère (82,8 %). La forte différenciation des saumons des lacs d'alevinage offre d'excellentes possibilités d'identification génétique des stocks fréquentant les divers réseaux fluviaux, mais la structure régionale relativement peu marquée limite les possibilités d'identification des stocks à l'échelle côtière. La variation génétique aux loci hautement structurés appuient l'hypothèse selon laquelle les populations canadiennes modernes proviennent de saumons rouges qui ont survécu à la glaciation du Wisconsin dans les refuges de Béring et du Colombia, et porte en outre à croire à l'existence de refuges côtiers en Colombie-Britannique.

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The sockeye salmon (*Oncorhynchus nerka*) is a commercially important anadromous salmonid whose oceanic distribution includes the North Pacific Ocean north of 46°N, and the Bering Sea south of 58°N. Spawning occurs primarily in rivers associated with nursery lakes along the coasts of Kamchatka and Alaska, including the Kuril and Aleutian Islands, Canada, and Washington (Forrester 1987). In Canada, sockeye spawn in more than 660 streams from the Alsek River in the Yukon Territory to the Fraser River in southern British Columbia (Department of Fisheries and Oceans, Canada, Salmon Escapement Database System; Serbic 1991). At least 80% of all Canadian sockeye originate in tributaries of large lakes in the Fraser, Skeena, and Nass river systems (Aro and Shepard 1967; Starr et al. 1984).

The stock assessment and management of sockeye populations are complicated by coastal fisheries that harvest mixtures of different populations. In addition, many sockeye populations cross international boundaries during their return migration and are harvested by multinational fisheries (e.g., Woodey 1987). Thus, effective stock identification techniques and knowledge of stock structure are necessary for negotiating international treaties and for managing sockeye populations. The feasibility of using biochemical genetic variation for defining salmon stocks and estimating stock contributions to mixed-stock fisheries has been demonstrated for most Pacific salmon (e.g., Allendorf and Utter 1979; Milner et al. 1985; Beacham et al. 1985a, 1985b) including sockeye salmon (Utter et al. 1973, 1984; Altukhov et al. 1975; Kirpichnikov and Ivanova 1977; Grant et al. 1980; Wilmot and Burger 1985; Withler 1987; Wood et al. 1987a, 1989; Kirpichnikov et al. 1990; Varnavskaya et al. 1994b).

In this paper, we document allozyme variation in Canadian sockeye populations surveyed from 1982–93 as part of a program to develop genetic stock identification procedures

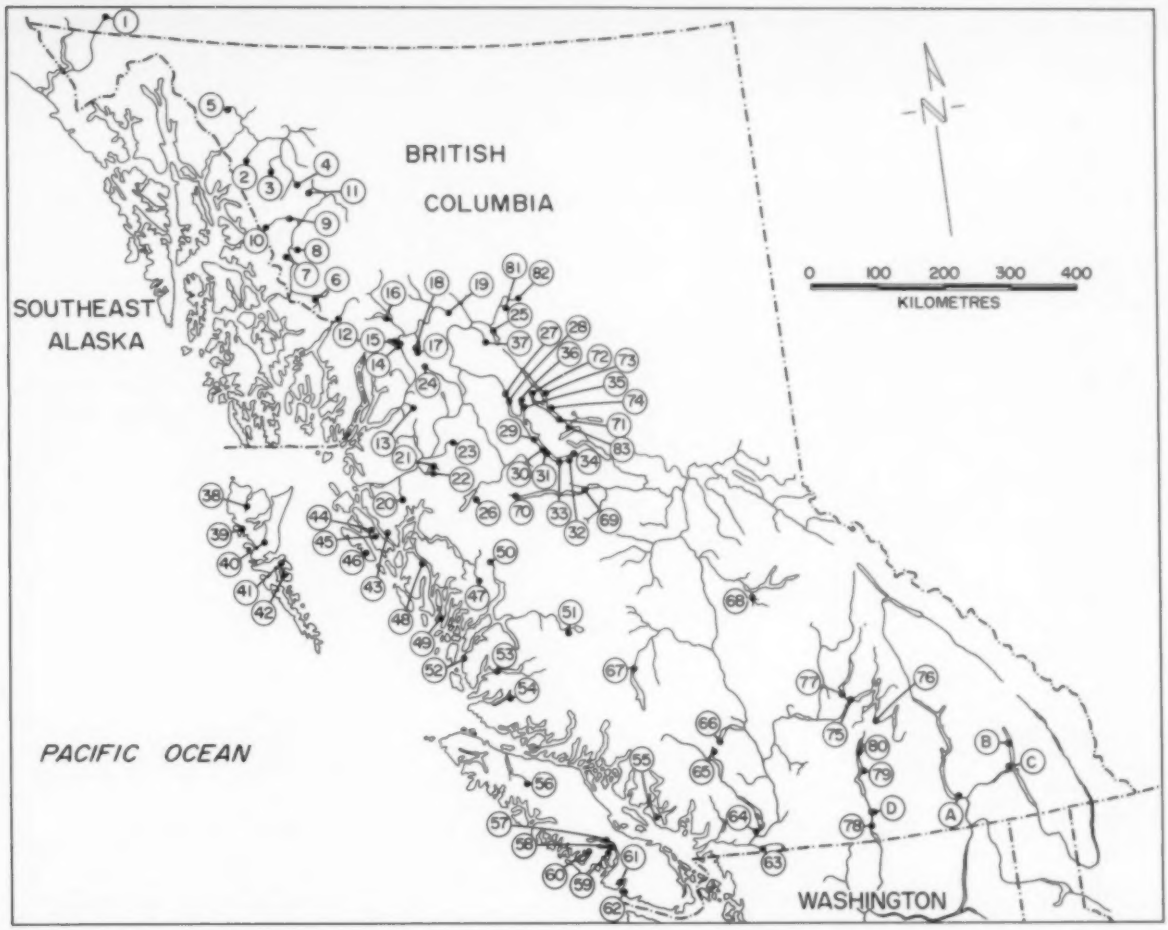

FIG. 1. Map showing sockeye collection sites. Numbers correspond to site numbers in Tables 1 and 3; letters denote the additional kokanee collection sites in Arrow Lake (A), Kootenay Lake (Meadow Creek (B), Redfish Creek (C)), and Skaha lake (D).

to support the Pacific Salmon Treaty between Canada and the United States. The survey included important sockeye populations in all major river systems and coastal lakes of western Canada. Collectively, these populations account for over 95% of Canadian sockeye production. We examine the geographical structuring of genetic variation in sockeye and discuss the potential of allozyme allele frequency data for stock composition analysis. We also speculate about the roles of homing behaviour, post-glacial dispersal routes and colonization events in shaping the peculiar genetic structure of sockeye salmon.

Methods

Sample Collection and Analysis

Sockeye were collected from 83 spawning sites throughout British Columbia and the Yukon Territory (Fig. 1, Table 1). To better characterize the Columbia River sockeye, two large samples (Nos. 79 and 80) of the nonanadromous ("kokanee") form of sockeye salmon from Okanagan Lake were included to complement the single sample (No. 78) of sockeye from Osoyoos Lake, the downstream portion of that drainage still accessible to anadromous fish. To examine post-glacial dispersal patterns, data from 10 additional sockeye or kokanee collections were used as examples of populations colonized from adjacent refuges in the Columbia River (McPhail and Lindsey 1986) and Beringia (Lindsey and McPhail 1986). These included kokanee from Arrow, Kootenay (Meadow Creek and Redfish Creek), and Skaha lakes in the Columbia River (data in Foote et al. 1989 except for *ADA-2** (monomorphic except in Arrow Lake where one **94/*100* genotype was detected in 100 fish) and *GPI-B1,2** (monomorphic)); sockeye from the Okanagan River and Wenatchee Lake in the Columbia River (data from Utter et al. 1984); sockeye from Gibraltar Creek and Woody Island in Iliamna Lake, Alaska (data in Bickham et al. 1995 except for *ADA-2** (monomorphic), *GPI-B1,2** (monomorphic), and *PGM-1** (**100* frequency 0.160 and 0.208 in 53 and 51 fish, respectively); and sockeye from Gavrushka Bay and Vichenkiya River in Kuril Lake, Kamchatka (data in Bickham et al. 1995 and Varnavskaya et al. 1994a).

Samples were typically obtained over a period of only 1 or 2 d near the peak of the spawning run. However, many sites were sampled in more than 1 yr (Table 1) and a few

TABLE 1. Summary of sockeye collections by nursery lake, major drainage, and river system. Site numbers correspond to numbers in Figs.1–3 and Table 3. Some data for years in parentheses published previously[a].

River system	Major drainage	Nursery lake	Collection site	Year
Alsek	Tatshenshini	Klukshu	1 Klukshu L	1992
Taku	Inklin	Little Trapper	2 Trapper L	1987, 92
		Litle Tatsamenie	3 Tatsamenie L	1985, 86, 92
		(none)	4 Hackett R	1985, 87
	Nakina	Kuthai	5 Kuthai L	1986, 87
Stikine	Iskut	(none)	6 Verrett R	(1983, 84, 85) 86, 89
	Stikine	Christina	7 Christina L	(1984, 85)
		(none)	8 Scud R	(1984, 85) 87
		(none)	9 Chutine R	(1984, 85)
		Chutine	10 Chutine L	(1984, 85) 87
		Tahltan	11 Tahltan L	(1982, 83, 85) 87
Unuk	Unuk	Border	12 Border L	(1987)
Nass	Lower Nass	(none)	13 Gingut Ck	(1987, 88)
		Meziadin	14 Tintina Ck	(1986)
			15 Hanna Ck	(1986)
	Bell-Irving	Bowser	16 Bowser L	(1984, 86, 87, 89)
	Upper Nass	Fred Wright	17 Bonney Ck	(1984, 86, 87)
			18 Kwinageese Ck	(1986, 87)
		Damdochax	19 Damdochax L	(1984, 86, 87, 89)
Skeena	Lower Skeena	Alastair	20 Alastair L	(1982, 83) 87, 88
		Lakelse	21 Williams Ck	(1982, 83) 87, 88
			22 Scully Ck	(1983) 88
		McDonnell	23 McDonnell L	1987, 88
	Upper Skeena	Swan	24 Swan L	1988
		Bear	25 Bear L	1987, 88
		Motass	37 Motass L	1987
		Sustut	81 Sustut R	1993
		Johanson	82 Johanson Ck	1993
	Bulkley	Morice	26 Nanika R	1988
	Babine	Babine	27 Lower Babine R	(1987)
			28 Upper Babine R	(1987)
			29 Fulton R/chan.	(1982, 83, 85, 87, 90)
			30 Pierre Ck	(1984, 85, 87, 88)
			31 Twain Ck	(1985, 87, 88)
			32 Four Mile Ck	(1982, 87, 88)
			33 Pinkut R/chan.	(1982, 83, 85, 87, 90)
			34 Grizzley Ck	(1987)
			35 Morrison R	(1988)
		Morrison	36 Tahlo Ck	(1987, 88)
Coastal	Queen Charlotte Islands	Eden	38 Eden L	(1982, 83)
		Mercer	39 Mercer L	(1982, 83)
		Yakoun	40 Yakoun L	(1982, 89) 93
		Skidegate	41 Skidegate L	(1989) 93
		Mathers	42 Mathers L	(1982)
	North mainland	Lowe	43 Lowe L	(1986)
		Devon	44 Devon L	(1985)
		Mikado	45 Mikado L	(1986)
		Banks	46 Banks L	(1986)
		Kitlope	47 Kitlope L	(1986)
		Canoona	48 Canoona L	(1986)
		Tankeeah	49 Tankeeah L	(1986)

(Fulton River, Pinkut Creek, and Iskut River) were also sampled near the beginning and end of spawning runs. Spawning sockeye were usually collected by tangle nets with 110-mm mesh or by beach seine; in some cases, sockeye were collected at weirs or by gaffs and pews (more details in Wood et al. 1987b; Rutherford et al. 1992).

TABLE 1. (*Concluded*)

River system	Major drainage	Nursery lake	Collection site	Year
	central-south mainland	Kimsquit	50 Kimsquit L	(1986)
		Tenas	51 Tenas L	(1985)
		Koeye	52 Koeye L	(1985)
		Owikeno	53 Owikeno L	(1982, 84, 89)
		Long	54 Long L	(1982, 84, 89)
		Sakinaw	55 Sakinaw L	(1988)
	Vancouver Island	Woss	56 Woss L	(1985)
		Great Central	57 Great Central L	(1983, 90)
		Sproat	58 Sproat L	(1983, 90)
		Henderson	59 Henderson L	(1983)
		Kennedy	60 Kennedy L	(1986)
		Hobiton	61 Hobiton L	(1987)
		Cheewhat	62 Cheewhat L	(1984)
Fraser	Lower Fraser	Cultus	63 Cultus L	(1985)
		Harrison	64 Weaver channel	1992
		Lillooet	65 Birkenhead R	1986, 92
	Upper Fraser	Anderson/Seton	66 Gates channel	1992
		Chilko	67 Chilko L	1992
		Quesnel	68 Horsefly R	(1985) 93
		Fraser	69 Stellako R	1992
		Francois	70 Nadina channel	1992
		Takla	71 Gluskie Ck	(1991)
			72 Dust Ck	(1988, 89, 91)
			73 Shale Ck	(1988, 89, 91)
			74 Narrows Ck	(1983, 85, 88, 91)
		Trembleur	83 Middle R	1993
	Thompson	Shuswap	75 Adams R	1990
			76 Shuswap R	(1983, 86) 90
		Adams	77 Sinmax Ck	1990
Columbia	Okanagan	Osoyoos	78 Okanagan R[b]	1989, 93
		Okanagan	79 Mission Ck	1989
			80 Okanagan L	1989

[a]Reference for previously published data: (1) Withler (1985): *LDH-B2** only for all sites sampled in 1982–83. (2) Quinn et al. (1987): sites 53, 54, 57–59 (1982–84). (3) Wood et al. (1987b): sites 6–11 (1983–85). (4) Rutherford et al. (1988): sites 60–62. (5) Wood et al. (1988): sites 29, 32, 33 (1982–83). (6) Foote et al. (1989): sites 29–31, 33 (1984–85); sites 63, 68, 74, 76 (1983–86). (7) Rutherford et al. (1992): sites 12, 38–58 (1982–1990). (8) Rutherford et al. (1994): sites 13–19 (1984–89). (9) Varnavskaya et al. (1994b): sites 27–36 (1987–90). (10) Wood and Foote (1994): sites 71–74 (1985–91).

[b]1993 sample collected at Wells Dam.

Liver, heart, retina, and skeletal muscle tissues were removed from freshly killed sockeye and stored in liquid nitrogen, on dry ice, or in domestic freezers at −20°C until they could be transferred to ultracold freezers for longer term storage. The tissues were analyzed by horizontal starch gel electrophoresis either at the Pacific Biological Station (1982–83), or under contract to Aqua-Life Diagnostics, Ltd., Nanaimo, B.C. (1984–86, 1988–93) and Helix-Biotech, Ltd., Vancouver, B.C. (1987) using procedures described by Aebersold et al. (1987) and C. Guthrie (Auke Bay Laboratory, Auke Bay, AK, personal communication).

Eighteen enzyme systems representing 35 loci (including 5 isoloci) were screened in most populations (Table 2). Two loci (*mAAT-1** and *PEP-LT** using the nomenclature of Shaklee et al. 1990) were added recently because they were reported to show useful variation in other sockeye populations (C. Guthrie, Auke Bay Laboratory, Auke Bay, AK, personal communication), and consequently have yet to be examined in many populations. The remaining 33 loci were not selected on a priori expectations of finding polymorphism; rather, they were all loci for which reliable electrophoretic techniques and scoring criteria were available when the study began in 1982. Sample sizes are listed in Table 3. We refer to the 33 nonselected loci screened in samples collected from 63 spawning sites in 1982–92 as the "complete set."

Data Analysis

Allele frequencies were determined by direct count for all loci except *PGM-1** where the frequency of the variant null allele was estimated as the square root of the null genotype frequency (assuming a Hardy–Weinberg distribution). Loci were defined as "highly polymorphic," "polymorphic," or monomorphic if the relative frequency of variant alleles (q) was $q > 0.05$, $0 < q \leq 0.05$, or $q = 0$, respectively. Genotype frequencies at all loci (excluding *PGM-1**) that were highly polymorphic within samples were examined for consistency with Hardy–Weinberg expectations using

TABLE 2. Enzymes and tissues used to investigate genetic variation. Tissues: E = eye; H = heart; L = liver; M = skeletal muscle. Buffers: AC = amine–citrate (Clayton and Tretiak 1972); RW = Tris, citric acid, lithium hydroxide, and boric acid (Ridgway et al. 1970); MF = Tris, boric acid), and EDTA, pH 8.5 (Markert and Faulhaber 1965).

Enzyme name	E.C. No.	Locus	Tissue	Buffer
Aconitate hydratase	4.2.1.3	*sAH**	L	AC
Adenosine deaminase	3.5.4.4	*ADA-2**	L	AC
Alanine aminotransferase	2.6.1.2	*ALAT**	M	MF
Aspartate aminotransferase	2.6.1.1	*mAAT-1**	M	AC
		*sAAT-1,2**	H	AC
		*sAAT-3**	E	AC
Dipeptidase	3.4.*.*	*PEPA**	E	MF
Glyceraldehyde-3-phosphate-dehydrogenase	1.2.1.12	*GAPDH-4**	E	AC
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1,2**	M	AC
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B1,2**	M	MF
Isocitrate dehydrogenase ($NADP^+$)	1.1.142	*mIDHP-1**	M	AC
		*mIDHP-2**	M	AC
		*sIDHP-1**	L	AC
		*sIDHP-2**	L	AC
Lactate dehydrogenase	1.1.1.27	*LDH-A1**	M	MF
		*LDH-A2**	M	MF
		*LDH-B1**	H	AC
		*LDH-B2**	L	RW
		*LDH-C**	E	MF
Leucyl-tyrosine peptidase	3.4.*.*	*PEP-LT**	M	MF
Malate dehydrogenase	1.1.137	*sMDH-A1,2**	L	AC
		*sMDH-B1,2**	M	AC
Malic enzyme ($NADP^+$)	1.1.1.40	*ME**	M	AC
		*sMEP-1**	M	AC
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	H	AC
Peptidase gly-leucyl	3.4.*.*	*PEPC**	E	MF
Phosphoglucomutase	5.4.2.2	*PGM-1**	H	AC
		*PGM-2**	M	MF
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M	AC
Superoxide dismutase	1.15.1.1	*sSOD**	L	RW

chi-square tests (BIOSYS release 1.7; Swofford and Selander 1981). Replicate samples from the same spawning site in different years, or at different times during the run, were pooled after comparing genotype frequencies at each locus using likelihood ratio chi-square tests. Some of these findings have already been published in more detail for some populations in the Stikine River (Wood et al. 1987b), the Nass River (Rutherford et al. 1994), the coastal region (Quinn et al. 1987; Wood et al. 1988; Rutherford et al. 1992), and in Babine, Shuswap, and Takla lakes (Foote et al. 1989; Wood and Foote 1995). Allele frequencies for seven samples collected in 1993 have been added to Table 1, but analyses were not revised to include the new data; allele frequencies in replicate 1993 samples did not differ significantly from those observed in previous years.

A hierarchical gene diversity analysis (Chakraborty 1980) was performed (using BIOSYS) to assess geographical structuring of genetic variation among the 63 spawning sites for which the complete set of loci was examined. The nested hierarchy included 8 "river systems," 20 "major drainages," 52 "nursery lakes," and 63 "spawning sites" (Table 1). The same allele frequency data were used to construct a neighbour-joining dendrogram (Saitou and Nei 1987) from Cavalli-Sforza and Edwards (1967) chord distances (using PHYLIP version 3.4; J. Felsenstein, University of Washington, Seattle, WA), and a UPGMA dendrogram (Sneath and Sokal 1973) from Nei (1978) unbiased genetic distances (using BIOSYS).

Likelihood ratio chi-square tests were performed to detect heterogeneity among samples from different sites within the same nursery lake. Spawning sites within a nursery lake were pooled if no significant difference between them was detected, thereby creating 57 distinct "populations." Average heterozygosity over 33 loci (as expected under Hardy–Weinberg; Nei 1978) was calculated for each population, and then averaged over various population groupings for comparisons among populations and regions.

The potential for identifying the 56 sockeye populations (kokanee excluded) was assessed by examining the cumulative effect of differences in genotype frequencies at the 14 highly polymorphic loci (Tables 3A and 3B) as revealed in a single-linkage similarity dendrogram constructed from log-likelihood ratio distances (Wood 1989). A principal components analysis was also performed on arcsine-transformed allele frequency data at seven loci (*PGM-1**, *ALAT**, *LDH-B2**, *PGM-2**, *PEPC**, *ADA-2**, and *GPI-B1,2**) available from the 57 populations and from four of the additional collections: Arrow Lake (kokanee), Iliamna Lake (Gibraltar Creek and Woody Island) and Kuril Lake (Vichenkiya River).

Results

Level of Polymorphism

Of the 33 nonselected loci examined initially, 14 (42%) were highly polymorphic and at least 10 (30%) were less

TABLE 3A. Frequencies of variant alleles at the most polymorphic loci ($q > 0.25$) and mean heterozygosity across 33 loci. Significant deviation from Hardy–Weinberg equilibrium denoted by # symbol.

River System	Site No.	Mean hetero-zygosity	ADA-2* (N)	*94	*107	ALAT* (N)	*91	*95	*108	LDH-B2* (N)	*115	*85	PEPC* (N)	*105	PGM-1* (N)	*NULL	PGM-2* (N)	*135	*166
ALSEK	1	0.040	76	0.000	0.000	76	0.026	0.230	0.000	76	0.079	0.007	76	0.000	75	0.600	75	0.160	0.000
TAKU	2	0.049	188	0.014	0.000	178	0.632	0.177	0.000	188	0.186	0.000	98	0.041	182	0.860	182	0.088	0.016
	3	0.046	268	0.000	0.000	170	0.444	0.300	0.000	270	0.156	0.000	156	0.008	267	0.860	269	0.106	0.037
	4	0.055	61	0.000	0.000	86	0.343	0.273	0.000	90	0.261	0.000	57	0.000	85	0.806	85	0.176	0.018
	5	0.051	175	0.000	0.000	171	0.444	0.444	0.000	174	0.296	0.000	168	0.080	162	0.890	173	0.020	0.000
STIKINE	6	0.049	304	0.000	0.000	229	0.410	0.212	0.004	699	0.112	0.004	216	0.028	479	0.761	691	0.192	0.008
	7	.	121	0.000	0.000	0	.	.	.	121	0.050	0.000	0	.	75	0.820	75	0.247	0.027
	8	0.048	204	0.000	0.000	86	0.390	0.180	0.000	#215	0.119	0.000	83	0.048	210	0.819	210	0.167	0.017
	9	.	110	0.000	0.000	0	.	.	.	112	0.134	0.000	0	.	109	0.789	109	0.179	0.014
	10	.	149	0.000	0.000	0	.	.	.	167	0.057	0.008	0	.	164	0.649	165	0.118	0.030
	11	0.056	99	0.000	0.000	99	0.323	0.439	0.000	400	0.574	0.001	74	0.081	384	0.889	397	0.043	0.005
UNUK	12	.	49	0.000	0.000	49	0.612	0.153	0.000	49	0.041	0.000	0	.	49	0.887	49	0.184	0.000
NASS	13	0.037	168	0.000	0.000	165	0.552	0.058	0.000	168	0.027	0.000	98	0.027	168	0.872	168	0.185	0.000
	14	0.040	100	0.000	0.000	95	0.584	0.042	0.037	99	0.510	0.000	100	0.005	100	0.910	100	0.080	0.000
	15	0.039	98	0.000	0.000	98	0.597	0.046	0.020	99	0.606	0.000	99	0.010	99	0.914	99	0.080	0.000
	16	0.044	256	0.000	0.000	164	0.549	0.168	0.008	261	0.228	0.000	199	0.060	260	0.896	254	0.067	0.000
	17	0.047	277	0.000	0.000	177	0.455	0.218	0.000	279	0.366	0.000	90	0.089	278	0.928	279	0.108	0.000
	18	0.048	196	0.000	0.000	193	0.544	0.187	0.010	199	0.188	0.000	97	0.010	200	0.820	200	0.183	0.000
	19	0.045	298	0.000	0.000	#195	0.692	0.179	0.013	#299	0.151	0.008	#197	0.071	298	0.866	298	0.188	0.005
SKEENA	20	0.049	150	0.000	0.000	150	0.241	0.198	0.000	350	0.000	0.000	75	0.000	340	0.871	350	0.366	0.004
	21	0.031	181	0.000	0.000	179	0.497	0.042	0.011	279	0.018	0.000	98	0.000	255	0.980	227	0.200	0.000
	22	0.031	77	0.000	0.000	77	0.370	0.019	0.045	127	0.020	0.000	77	0.006	99	0.985	127	0.248	0.000
	23	0.052	162	0.000	0.000	156	0.608	0.106	0.000	#162	0.114	0.000	75	0.120	162	0.691	162	0.157	0.000
	24	0.027	100	0.000	0.000	100	0.600	0.080	0.070	100	0.025	0.000	100	0.000	100	0.980	100	0.140	0.000
	25	0.043	148	0.000	0.000	145	0.576	0.110	0.021	152	0.148	0.000	#71	0.056	152	0.908	143	0.073	0.045
	81	0.046	98	0.000	0.000	98	0.598	0.296	0.000	98	0.129	0.000	98	0.000	98	0.796	98	0.097	0.000
	82	0.037	60	0.000	0.000	60	0.550	0.342	0.000	60	0.042	0.000	59	0.000	60	0.767	60	0.100	0.000
	26	0.088	75	0.000	0.000	74	0.764	0.108	0.000	75	0.000	0.120	75	0.100	75	0.888	75	0.088	0.000
	27	.	97	0.010	0.000	82	0.457	0.061	0.006	99	0.000	0.015	0	.	98	0.760	99	0.192	0.000
	28	.	98	0.000	0.000	94	0.457	0.085	0.021	98	0.015	0.081	0	.	98	0.852	98	0.209	0.010
	29	0.043	491	0.001	0.000	698	0.501	0.092	0.019	1192	0.011	0.018	88	0.076	1114	0.880	1176	0.219	0.000
	30	0.042	275	0.000	0.000	258	0.459	0.088	0.002	370	0.015	0.018	171	0.082	373	0.798	373	0.239	0.000
	31	0.044	300	0.000	0.000	195	0.477	0.072	0.000	308	0.005	0.020	100	0.090	308	0.902	308	0.218	0.000
	32	0.044	163	0.000	0.000	163	0.451	0.089	0.008	285	0.015	0.048	73	0.041	280	0.807	285	0.234	0.002
	33	0.042	396	0.000	0.000	595	0.582	0.060	0.014	1049	0.010	0.020	50	0.050	1032	0.791	1044	0.242	0.000
	34	.	78	0.000	0.000	77	0.494	0.045	0.000	78	0.006	0.006	0	.	78	0.776	78	0.256	0.000
	35	0.042	76	0.000	0.000	75	0.467	0.100	0.013	76	0.013	0.013	76	0.072	76	0.886	76	0.191	0.000
	36	.	163	0.000	0.000	164	0.476	0.055	0.000	164	0.006	0.015	85	0.058	164	0.857	164	0.235	0.000
	37	.	78	0.000	0.000	46	0.446	0.120	0.088	74	0.074	0.000	0	.	74	0.689	74	0.101	0.000
COASTAL	38	.	0	.	.	0	.	.	.	76	0.007	0.000	0	.	76	0.757	76	0.257	0.000
	39	.	0	.	.	0	.	.	.	149	0.356	0.000	0	.	140	0.907	149	0.438	0.000
	40	0.086	81	0.000	0.000	81	0.469	0.000	0.000	181	0.000	0.000	81	0.049	180	0.904	181	0.225	0.000
	41	0.088	49	0.000	0.000	49	0.510	0.010	0.000	49	0.000	0.000	49	0.296	49	0.959	49	0.041	0.000
	42	.	0	.	.	0	.	.	.	49	0.000	0.000	0	.	50	0.910	50	0.080	0.000
	43	0.081	92	0.000	0.000	98	0.790	0.145	0.000	92	0.000	0.000	91	0.044	89	0.916	89	0.264	0.000
	44	0.080	100	0.000	0.000	92	0.435	0.179	0.000	100	0.080	0.000	94	0.082	98	0.980	98	0.010	0.000
	45	0.086	100	0.000	0.000	99	0.379	0.222	0.000	100	0.000	0.000	96	0.089	100	0.970	100	0.040	0.000
	46	0.047	100	0.000	0.000	100	0.485	0.300	0.000	100	0.065	0.000	96	0.081	100	0.845	100	0.165	0.000
	47	0.086	45	0.000	0.000	42	0.345	0.071	0.086	45	0.011	0.000	45	0.000	45	0.800	45	0.156	0.000
	48	0.082	100	0.000	0.000	100	0.205	0.655	0.000	100	0.000	0.000	100	0.005	100	0.550	100	0.020	0.000
	49	0.087	100	0.000	0.000	100	0.495	0.295	0.000	100	0.045	0.000	96	0.086	100	0.885	100	0.080	0.000
	50	0.082	80	0.000	0.000	80	0.656	0.050	0.063	81	0.000	0.000	80	0.088	79	0.658	79	0.006	0.000
	51	0.084	97	0.000	0.000	95	0.468	0.011	0.000	100	0.055	0.005	100	0.000	100	0.800	100	0.085	0.000
	52	.	99	0.000	0.000	0	.	.	.	100	0.005	0.000	0	.	100	0.895	100	0.130	0.080
	53	0.045	198	0.023	0.000	100	0.480	0.165	0.060	300	0.028	0.000	99	0.020	300	0.738	298	0.148	0.000
	54	0.047	199	0.013	0.000	100	0.210	0.225	0.015	284	0.014	0.000	101	0.025	288	0.486	284	0.187	0.000
	55	0.028	88	0.000	0.000	92	0.726	0.043	0.000	88	0.000	0.000	88	0.000	88	0.157	88	0.024	0.000
	56	0.023	100	0.000	0.000	100	0.475	0.025	0.000	100	0.000	0.000	98	0.005	99	0.990	100	0.105	0.000
	57	0.048	100	0.005	0.000	100	0.550	0.040	0.000	248	0.000	0.000	100	0.085	210	0.752	235	0.138	0.000
	58	0.051	50	0.080	0.000	49	0.622	0.051	0.000	233	0.002	0.000	50	0.080	181	0.605	232	0.192	0.000
	59	.	0	.	.	0	.	.	.	154	0.000	0.000	0	.	138	0.964	149	0.023	0.000
	60	0.024	91	0.000	0.000	91	0.588	0.071	0.000	91	0.000	0.000	91	0.066	89	0.961	89	0.022	0.000
	61	.	68	0.000	0.000	99	0.585	0.015	0.000	100	0.000	0.000	86	0.110	100	0.900	100	0.010	0.000
	62	0.087	99	0.000	0.000	97	0.660	0.098	0.000	100	0.000	0.000	100	0.005	100	0.625	100	0.185	0.000
FRASER	63	.	78	0.000	0.000	0	.	.	.	99	0.000	0.000	0	.	58	0.858	95	0.163	0.000
	64	0.042	100	0.050	0.000	100	0.500	0.095	0.000	100	0.000	0.000	100	0.000	100	0.780	100	0.225	0.015
	65	0.057	249	0.018	0.000	244	0.547	0.119	0.000	249	0.000	0.000	240	0.002	242	0.748	242	0.184	0.000
	66	0.042	99	0.116	0.000	99	0.207	0.020	0.000	99	0.000	0.000	99	0.126	99	0.141	99	0.131	0.000
	67	0.046	99	0.080	0.000	99	0.237	0.282	0.000	99	0.000	0.000	99	0.000	99	0.404	99	0.217	0.000
	68	0.050	98	0.005	0.000	94	0.330	0.101	0.000	100	0.000	0.000	95	0.005	93	0.376	100	0.195	0.000
	69	0.086	100	0.010	0.000	100	0.370	0.005	0.000	100	0.000	0.000	100	0.000	98	0.265	98	0.148	0.000
	70	0.029	98	0.005	0.000	98	0.163	0.010	0.000	98	0.000	0.000	98	0.005	98	0.204	98	0.036	0.000
	71	0.031	42	0.000	0.000	42	0.071	0.036	0.000	42	0.000	0.000	42	0.000	42	0.440	42	0.179	0.000
	72	0.040	67	0.000	0.000	101	0.188	0.074	0.010	67	0.000	0.067	67	0.000	66	0.278	100	0.185	0.000
	73	0.035	101	0.000	0.000	130	0.181	0.042	0.000	101	0.000	0.000	101	0.000	101	0.371	130	0.177	0.000
	74	0.035	190	0.000	0.000	288	0.148	0.042	0.000	366	0.000	0.000	289	0.000	367	0.437	368	0.185	0.000
	83	0.052	90	0.011	0.000	90	0.111	0.050	0.000	90	0.000	0.000	90	0.006	90	0.394	90	0.183	0.000
	75	0.042	100	0.045	0.000	100	0.195	0.055	0.015	100	0.005	0.000	100	0.000	100	0.265	100	0.165	0.000
	76	0.042	101	0.079	0.000	#320	0.188	0.108	0.078	342	0.006	0.000	242	0.000	343	0.465	341	0.126	0.000
	77	0.043	51	0.265	0.000	51	0.167	0.010	0.000	50	0.000	0.000	51	0.000	51	0.848	51	0.098	0.000
COLUMBIA	78	0.054	26	0.019	0.038	26	0.231	0.173	0.212	20	0.000	0.000	26	0.000	26	0.519	26	0.096	0.000
	79	0.051	102	0.206	0.029	102	0.446	0.201	0.083	102	0.000	0.000	102	0.000	102	0.858	102	0.127	0.000
	80	0.048	100	0.180	0.000	100	0.515	0.195	0.090	100	0.000	0.000	100	0.015	100	0.855	100	0.150	0.000

polymorphic, conservatively assuming that variation existed at only one locus of each pair of isoloci (Table 3). The two loci added because they had been reported highly polymorphic elsewhere (*mAAT-1** and *PEP-LT**), were also highly polymorphic in some Canadian sockeye populations, making a total of 16 highly polymorphic loci. Only three loci (*PGM-1**, *PGM-2**, and *ALAT**) were polymorphic in all populations examined. Two of these loci also showed the greatest variability among populations, with the frequency of *PGM-1*-100* ranging from 0.01 to 0.86, and *ALAT*100* ranging from 0.07 to 0.89. No two populations were fixed for alternative alleles at any locus. Five additional loci were potentially very

TABLE 3B. Frequencies of variant alleles at other highly polymorphic loci ($0.05 < q \leq 0.25$).

River System	Site No.	sAAT-1,2* (N)	*83	sAAT-3* (N)	*117	GPI-B1,2* (N)	*182	*20	sIDHP-2* (N)	*118	sMDH-B1,2* (N)	*85	*105	sMEP-1* (N)	*63	MPI* (N)	*95	PGDH* (N)	*94	*108
ALSEK	1	75	0.000	76	0.000	76	0.007	0.000	76	0.000	76	0.000	0.000	76	0.000	75	0.000	76	0.000	0.000
TAKU	2	102	0.049	103	0.046	99	0.000	0.000	103	0.000	103	0.000	0.000	103	0.000	102	0.000	103	0.000	0.000
	3	270	0.000	269	0.054	200	0.000	0.000	269	0.000	270	0.000	0.000	268	0.000	270	0.000	270	0.000	0.000
	4	86	0.006	88	0.068	61	0.000	0.000	91	0.000	90	0.000	0.000	88	0.000	89	0.000	90	0.000	0.000
	5	171	0.000	168	0.107	74	0.000	0.000	175	0.008	175	0.000	0.000	175	0.006	173	0.000	175	0.000	0.000
STIKINE	6	422	0.000	396	0.006	449	0.000	0.001	470	0.000	474	0.007	0.000	408	0.000	469	0.000	472	0.000	0.000
	7	76	0.020	114	0.009	119	0.000	0.000	119	0.004	122	0.000	0.000	122	0.000	122	0.000	122	0.000	0.000
	8	215	0.000	198	0.018	117	0.000	0.004	214	0.007	214	0.002	0.000	213	0.000	211	0.000	210	0.000	0.000
	9	110	0.005	105	0.019	112	0.000	0.004	110	0.018	111	0.009	0.000	112	0.000	112	0.000	112	0.000	0.000
	10	166	0.000	152	0.046	148	0.000	0.000	164	0.000	167	0.000	0.000	167	0.000	166	0.000	165	0.000	0.000
	11	335	0.000	291	0.125	298	0.000	0.000	299	0.000	299	0.000	0.000	277	0.000	300	0.000	307	0.000	0.000
UNUK	12	49	0.000	0	.	0	.	.	0	.	49	0.000	0.000	49	0.000	49	0.000	49	0.000	0.000
NASS	13	168	0.000	167	0.000	93	0.000	0.000	168	0.000	168	0.000	0.000	168	0.000	168	0.000	168	0.000	0.000
	14	100	0.000	99	0.015	100	0.000	0.000	100	0.000	99	0.000	0.000	100	0.000	98	0.000	100	0.000	0.000
	15	99	0.000	97	0.021	94	0.000	0.000	99	0.000	98	0.000	0.000	98	0.000	99	0.000	98	0.000	0.000
	16	261	0.000	226	0.040	178	0.000	0.000	262	0.000	254	0.000	0.000	262	0.000	259	0.000	259	0.000	0.000
	17	278	0.000	275	0.007	198	0.000	0.000	280	0.000	280	0.000	0.000	280	0.000	278	0.000	279	0.000	0.002
	18	200	0.000	169	0.018	99	0.000	0.000	199	0.000	200	0.000	0.000	197	0.000	199	0.000	200	0.000	0.000
	19	298	0.000	256	0.045	197	0.018	0.000	296	0.000	298	0.000	0.000	299	0.000	297	0.000	296	0.000	0.000
SKEENA	20	141	0.195	147	0.000	254	0.000	0.000	349	0.004	350	0.000	0.000	350	0.000	249	0.000	350	0.000	0.000
	21	191	0.009	170	0.000	186	0.000	0.000	276	0.014	278	0.000	0.000	278	0.000	222	0.000	278	0.000	0.000
	22	77	0.006	77	0.000	123	0.000	0.000	127	0.008	127	0.000	0.000	122	0.000	77	0.000	122	0.000	0.000
	23	162	0.000	147	0.027	74	0.000	0.000	162	0.000	162	0.000	0.000	162	0.000	162	0.000	162	0.000	0.000
	24	100	0.000	100	0.000	100	0.005	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	0.000
	25	149	0.000	149	0.000	70	0.057	0.000	152	0.000	152	0.000	0.000	152	0.000	152	0.000	145	0.000	0.000
	81	93	0.000	93	0.000	93	0.000	0.000	93	0.000	93	0.000	0.000	93	0.000	93	0.000	93	0.000	0.000
	82	60	0.000	59	0.000	59	0.000	0.000	60	0.000	60	0.000	0.000	60	0.000	60	0.000	60	0.000	0.000
	26	75	0.000	75	0.000	75	0.000	0.000	75	0.000	75	0.000	0.000	75	0.000	75	0.000	74	0.068	0.000
	27	97	0.000	91	0.000	0	.	.	99	0.000	99	0.000	0.000	99	0.000	98	0.000	98	0.000	0.000
	28	98	0.000	91	0.000	0	.	.	98	0.000	99	0.000	0.000	98	0.000	98	0.000	98	0.000	0.000
	29	486	0.000	175	0.000	494	0.000	0.000	384	0.001	376	0.000	0.000	313	0.000	333	0.000	377	0.000	0.000
	30	271	0.000	174	0.000	175	0.000	0.000	178	0.000	179	0.000	0.000	179	0.000	178	0.000	175	0.000	0.000
	31	302	0.000	193	0.000	203	0.000	0.000	199	0.000	198	0.000	0.000	200	0.000	199	0.000	197	0.008	0.000
	32	162	0.000	163	0.000	103	0.000	0.000	235	0.000	235	0.000	0.000	230	0.000	233	0.000	235	0.000	0.000
	33	396	0.000	199	0.000	381	0.000	0.000	450	0.000	447	0.000	0.000	449	0.000	277	0.000	440	0.000	0.000
	34	78	0.000	77	0.000	0	.	.	78	0.000	78	0.000	0.000	78	0.000	78	0.000	78	0.000	0.000
	35	76	0.000	76	0.000	76	0.000	0.000	76	0.000	76	0.000	0.000	76	0.000	76	0.000	76	0.000	0.000
	36	164	0.000	163	0.000	0	.	.	164	0.000	164	0.000	0.000	164	0.000	164	0.000	164	0.000	0.000
	37	74	0.000	74	0.000	0	.	.	74	0.000	74	0.000	0.000	74	0.000	74	0.000	74	0.000	0.000
COASTAL	38	0	.	0	.	74	0.000	0.027	76	0.020	76	0.013	0.000	76	0.000	75	0.000	76	0.000	0.000
	39	0	.	0	.	134	0.000	0.000	147	0.007	150	0.007	0.000	149	0.000	149	0.000	150	0.000	0.000
	40	81	0.000	80	0.000	125	0.000	0.000	130	0.008	131	0.004	0.000	131	0.000	122	0.000	131	0.000	0.000
	41	49	0.000	49	0.000	49	0.000	0.000	49	0.000	49	0.000	0.000	49	0.000	49	0.000	49	0.000	0.000
	42	0	.	0	.	47	0.000	0.000	50	0.010	50	0.000	0.000	50	0.000	49	0.000	50	0.000	0.000
	43	89	0.000	92	0.000	90	0.000	0.000	92	0.000	92	0.000	0.000	92	0.000	89	0.000	92	0.000	0.000
	44	100	0.000	100	0.000	90	0.000	0.000	100	0.000	100	0.005	0.000	100	0.005	97	0.067	98	0.000	0.000
	45	100	0.005	97	0.000	100	0.000	0.000	100	0.000	100	0.010	0.000	100	0.005	100	0.095	100	0.000	0.000
	46	100	0.005	98	0.000	100	0.000	0.000	100	0.000	100	0.065	0.000	100	0.000	100	0.000	100	0.000	0.000
	47	45	0.000	42	0.000	39	0.000	0.000	45	0.000	45	0.000	0.000	42	0.000	45	0.000	45	0.000	0.000
	48	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	0.000
	49	100	0.005	100	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	0.000
	50	79	0.000	81	0.000	79	0.000	0.000	81	0.000	80	0.000	0.000	80	0.000	79	0.000	81	0.000	0.000
	51	100	0.000	100	0.000	98	0.000	0.000	100	0.000	100	0.000	0.000	99	0.000	100	0.000	100	0.000	0.000
	52	100	0.000	100	0.000	98	0.000	0.000	98	0.000	100	0.000	0.000	98	0.000	100	0.000	100	0.000	0.000
	53	200	0.000	199	0.000	298	0.000	0.000	295	0.008	299	0.000	0.000	300	0.000	299	0.000	298	0.000	0.000
	54	200	0.000	195	0.000	266	0.000	0.000	284	0.000	286	0.012	0.003	284	0.000	283	0.000	284	0.000	0.000
	55	83	0.000	83	0.000	83	0.000	0.000	83	0.000	83	0.000	0.000	83	0.000	83	0.000	83	0.000	0.000
	56	98	0.000	92	0.000	97	0.000	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	0.000
	57	99	0.005	100	0.000	176	0.000	0.000	243	0.016	243	0.025	0.000	236	0.000	191	0.000	236	0.091	0.000
	58	50	0.000	50	0.000	178	0.000	0.000	233	0.000	230	0.007	0.000	233	0.000	143	0.000	233	0.144	0.000
	59	0	.	0	.	111	0.014	0.000	154	0.000	124	0.040	0.000	154	0.000	118	0.000	104	0.000	0.000
	60	91	0.000	90	0.000	90	0.000	0.000	91	0.000	91	0.000	0.000	91	0.000	91	0.000	91	0.000	0.000
	61	98	0.000	95	0.000	0	.	.	97	0.031	100	0.005	0.000	100	0.000	100	0.000	100	0.015	0.000
	62	100	0.000	0	.	99	0.000	0.000	99	0.010	99	0.005	0.000	99	0.000	100	0.000	99	0.000	0.000
FRASER	63	96	0.000	95	0.000	97	0.000	0.000	99	0.000	99	0.000	0.000	96	0.000	98	0.000	74	0.000	0.000
	64	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.010	0.000	100	0.000	100	0.000	100	0.000	0.000
	65	243	0.000	228	0.000	244	0.180	0.000	159	0.138	249	0.012	0.000	125	0.004	244	0.000	249	0.000	0.000
	66	99	0.000	99	0.000	99	0.056	0.000	99	0.000	99	0.000	0.000	99	0.000	99	0.000	99	0.000	0.000
	67	99	0.000	99	0.000	99	0.005	0.000	99	0.000	99	0.000	0.000	99	0.000	99	0.000	99	0.000	0.000
	68	99	0.000	94	0.000	95	0.174	0.000	98	0.000	100	0.000	0.000	100	0.000	96	0.000	100	0.000	0.000
	69	98	0.000	100	0.000	100	0.020	0.000	100	0.000	100	0.000	0.000	100	0.015	98	0.000	100	0.000	0.000
	70	98	0.000	98	0.000	98	0.005	0.000	98	0.000	98	0.000	0.000	98	0.097	98	0.000	98	0.000	0.000
	71	42	0.000	42	0.000	42	0.000	0.000	42	0.000	42	0.000	0.000	42	0.000	42	0.000	42	0.000	0.000
	72	66	0.000	67	0.000	101	0.005	0.000	67	0.000	101	0.000	0.000	101	0.000	66	0.008	101	0.000	0.000
	73	101	0.000	101	0.000	130	0.015	0.000	101	0.000	130	0.000	0.000	130	0.000	101	0.000	130	0.000	0.000
	74	371	0.000	189	0.000	290	0.007	0.000	189	0.000	190	0.000	0.000	262	0.002	189	0.000	190	0.000	0.000
	83	90	0.000	90	0.000	90	0.061	0.000	90	0.006	90	0.000	0.000	90	0.006	90	0.000	90	0.000	0.000
	75	100	0.000	100	0.000	100	0.050	0.020	100	0.000	100	0.000	0.005	100	0.010	100	0.000	100	0.000	0.000
	76	339	0.000	242	0.000	243	0.014	0.000	242	0.004	101	0.000	0.000	101	0.000	245	0.000	247	0.000	0.000
	77	51	0.000	51	0.000	51	0.020	0.000	51	0.000	51	0.000	0.010	51	0.000	51	0.000	51	0.000	0.000
COLUMBIA	78	26	0.000	26	0.000	26	0.096	0.038	26	0.000	20	0.000	0.000	26	0.000	26	0.000	26	0.000	0.000
	79	102	0.000	102	0.000	102	0.000	0.000	102	0.000	102	0.010	0.000	102	0.000	102	0.000	102	0.000	0.000
	80	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.010	0.000	100	0.000	100	0.000	100	0.000	0.000

useful for genetic stock identification: *mAAT-1** (*-100 allele frequency range 0.36–1.0), *LDH-B2** (0.43–1.0), *PGM-2** (0.57–1.0), *PEPC** (0.70–1.0), and *ADA-2** (0.74–1.0). Common allele frequencies at other loci were never less than 0.80.

Variation Within Populations

Genotype frequency distributions within samples generally conformed to Hardy–Weinberg expectations for highly polymorphic loci that could be tested. Eight of 225 tests deviated significantly after allowing for multiple comparisons ($p < 0.0003$), and these occurred in 6 different collections (indicated in Table 3); 14 of the remaining tests deviated at $p < 0.05$, but 11 such deviations would be expected by chance. Genotype frequencies were remarkably stable from year to year with only 38 of 615 comparisons at 4 loci (*PGM-1**, *PGM-2**, *LDH-B2**, and *ALAT**) revealing

TABLE 3C. Frequencies of variant alleles at less polymorphic loci ($0 < q \leq 0.05$).

River System	Site No.	*AH* (N)	*117	*85	G3PDH-1* (N)	*150	*IDHP-1* (N)	*64	*IDHP-1* (N)	*82	LDH-B1* (N)	*123	*90	LDH-C* (N)	*92	*MDH-A1,2* (N)	*147	ME* (N)	*85	PEPA* (N)	*106	*SOD* (N)	*145	*84
ALSEK	1	76	0.000	0.000	76	0.000	76	0.000	76	0.000	75	0.000	0.000	75	0.000	76	0.000	76	0.000	76	0.000	76	0.000	0.000
TAKU	2	183	0.000	0.000	183	0.000	183	0.000	183	0.000	182	0.000	0.000	182	0.008	188	0.000	183	0.000	98	0.000	183	0.000	0.000
	3	257	0.000	0.000	268	0.000	269	0.000	269	0.000	269	0.000	0.000	264	0.000	270	0.000	268	0.000	156	0.000	270	0.002	0.000
	4	86	0.000	0.000	89	0.000	88	0.000	91	0.000	90	0.000	0.000	91	0.000	90	0.000	88	0.000	57	0.000	90	0.000	0.000
	5	173	0.029	0.000	175	0.000	175	0.000	175	0.000	171	0.000	0.000	173	0.000	173	0.000	175	0.006	168	0.000	174	0.000	0.000
STIKINE	6	391	0.000	0.000	449	0.007	300	0.000	470	0.000	412	0.000	0.000	385	0.000	418	0.000	408	0.000	216	0.000	402	0.000	0.000
	7	118	0.000	0.000	122	0.000	122	0.004	119	0.000	121	0.004	0.000	121	0.000	119	0.000	122	0.000	0	.	119	0.000	0.000
	8	188	0.000	0.000	209	0.000	211	0.000	214	0.000	215	0.000	0.000	201	0.000	204	0.000	218	0.000	83	0.000	214	0.000	0.000
	9	105	0.000	0.000	111	0.000	112	0.000	110	0.000	112	0.000	0.000	110	0.000	112	0.000	112	0.000	0	.	112	0.000	0.000
	10	153	0.000	0.000	167	0.000	167	0.000	164	0.000	166	0.000	0.000	152	0.000	166	0.000	167	0.000	0	.	157	0.000	0.000
	11	229	0.000	0.000	298	0.000	98	0.000	300	0.000	279	0.000	0.000	298	0.000	269	0.000	277	0.000	74	0.000	274	0.000	0.000
UNUK	12	49	0.000	0.000	49	0.000	49	0.000	0	.	49	0.000	0.000	0	.	49	0.000	49	0.000	0	.	49	0.000	0.000
NASS	13	168	0.000	0.000	169	0.030	168	0.000	168	0.000	168	0.000	0.000	168	0.000	168	0.000	168	0.000	93	0.005	168	0.000	0.000
	14	98	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000
	15	99	0.000	0.000	98	0.000	98	0.000	99	0.000	99	0.000	0.000	96	0.000	99	0.000	98	0.000	99	0.000	99	0.000	0.000
	16	261	0.000	0.000	257	0.000	256	0.000	262	0.000	261	0.000	0.000	204	0.000	262	0.000	262	0.000	199	0.000	258	0.000	0.000
	17	275	0.000	0.000	278	0.000	280	0.000	280	0.000	278	0.000	0.000	255	0.000	277	0.000	280	0.000	90	0.000	276	0.000	0.000
	18	200	0.002	0.000	195	0.000	199	0.000	199	0.000	200	0.000	0.000	147	0.000	200	0.000	197	0.000	97	0.000	199	0.000	0.000
	19	284	0.000	0.000	299	0.000	298	0.000	296	0.000	298	0.000	0.000	244	0.000	299	0.000	299	0.000	197	0.008	296	0.000	0.000
SKEENA	20	157	0.010	0.000	346	0.000	150	0.000	349	0.001	274	0.000	0.000	228	0.000	350	0.000	350	0.000	75	0.000	150	0.000	0.000
	21	180	0.000	0.000	275	0.004	91	0.000	276	0.000	222	0.000	0.000	264	0.002	279	0.000	278	0.000	98	0.000	181	0.000	0.000
	22	77	0.000	0.000	121	0.000	77	0.000	127	0.000	77	0.000	0.000	77	0.000	127	0.000	122	0.000	77	0.000	77	0.000	0.000
	23	161	0.000	0.000	162	0.000	162	0.000	162	0.000	162	0.000	0.000	151	0.000	162	0.000	162	0.000	75	0.000	161	0.000	0.000
	24	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000
	25	148	0.000	0.000	149	0.000	152	0.000	152	0.000	152	0.000	0.000	148	0.000	152	0.000	152	0.000	71	0.000	150	0.000	0.000
	91	93	0.000	0.000	93	0.000	93	0.000	93	0.000	93	0.000	0.000	93	0.000	93	0.000	93	0.000	93	0.000	93	0.000	0.000
	92	60	0.000	0.000	60	0.000	60	0.000	60	0.000	60	0.000	0.000	59	0.000	60	0.000	60	0.000	59	0.000	60	0.000	0.000
	26	75	0.000	0.000	75	0.000	75	0.000	75	0.000	75	0.000	0.000	75	0.000	75	0.000	75	0.000	75	0.007	75	0.000	0.000
	27	99	0.000	0.000	96	0.000	99	0.000	99	0.000	98	0.000	0.000	83	0.000	99	0.000	99	0.000	0	.	99	0.000	0.000
	28	98	0.000	0.000	98	0.000	98	0.000	98	0.000	98	0.000	0.000	87	0.000	98	0.000	98	0.000	0	.	98	0.000	0.000
	29	193	0.000	0.000	317	0.000	193	0.000	384	0.000	369	0.003	0.000	339	0.003	386	0.000	313	0.000	83	0.000	491	0.000	0.000
	30	178	0.003	0.000	179	0.000	179	0.000	178	0.000	179	0.003	0.000	143	0.000	179	0.000	179	0.000	171	0.000	177	0.000	0.000
	31	199	0.000	0.000	198	0.000	194	0.000	199	0.000	200	0.000	0.000	180	0.000	200	0.000	200	0.000	100	0.000	202	0.000	0.000
	32	164	0.000	0.000	234	0.000	164	0.000	235	0.000	233	0.000	0.000	208	0.005	235	0.002	230	0.000	73	0.000	164	0.000	0.000
	33	196	0.000	0.000	450	0.000	200	0.000	450	0.000	338	0.000	0.000	312	0.000	450	0.000	449	0.000	50	0.000	400	0.000	0.000
	34	77	0.000	0.000	76	0.000	78	0.000	78	0.000	78	0.000	0.000	45	0.000	78	0.000	78	0.000	0	.	78	0.000	0.000
	35	76	0.000	0.000	76	0.000	76	0.000	76	0.000	76	0.000	0.000	76	0.000	76	0.000	76	0.000	76	0.000	76	0.000	0.000
	36	164	0.000	0.000	164	0.000	164	0.000	164	0.000	164	0.000	0.000	164	0.000	164	0.000	164	0.000	85	0.000	164	0.000	0.000
	37	72	0.000	0.000	74	0.000	74	0.000	74	0.000	74	0.000	0.000	74	0.000	74	0.000	74	0.000	0	.	74	0.000	0.000
COASTAL	38	0	.	.	49	0.000	0	.	76	0.000	76	0.000	0.000	74	0.000	76	0.000	76	0.000	0	.	0	.	.
	39	0	.	.	129	0.000	0	.	147	0.000	149	0.000	0.000	146	0.000	150	0.000	149	0.000	0	.	0	.	.
	40	81	0.000	0.000	130	0.000	81	0.000	130	0.000	131	0.000	0.000	100	0.000	131	0.000	131	0.000	81	0.019	81	0.000	0.000
	41	49	0.000	0.000	49	0.000	49	0.000	49	0.000	49	0.000	0.000	49	0.000	49	0.000	49	0.000	49	0.000	49	0.000	0.000
	42	0	.	.	28	0.000	0	.	50	0.000	49	0.000	0.000	48	0.000	50	0.000	50	0.000	0	.	0	.	.
	43	90	0.008	0.028	92	0.000	90	0.000	92	0.000	89	0.000	0.000	90	0.000	92	0.000	92	0.000	92	0.000	85	0.000	0.000
	44	99	0.000	0.005	100	0.000	100	0.000	100	0.000	100	0.000	0.000	99	0.000	100	0.000	100	0.000	94	0.000	100	0.000	0.000
	45	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.005	98	0.000	100	0.000	0.000
	46	100	0.005	0.025	100	0.000	100	0.000	100	0.000	100	0.000	0.000	98	0.000	100	0.000	100	0.000	98	0.000	100	0.000	0.000
	47	45	0.000	0.000	45	0.000	45	0.000	45	0.000	45	0.000	0.000	45	0.000	45	0.000	42	0.000	45	0.000	45	0.000	0.000
	48	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000
	49	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	98	0.000	100	0.000	0.000
	50	81	0.000	0.000	80	0.000	81	0.000	81	0.000	79	0.000	0.000	81	0.000	81	0.000	80	0.000	80	0.000	81	0.000	0.000
	51	100	0.000	0.000	98	0.000	100	0.000	100	0.000	100	0.000	0.000	98	0.000	100	0.000	99	0.000	100	0.000	100	0.000	0.000
	52	96	0.000	0.000	99	0.000	100	0.000	98	0.000	100	0.000	0.000	100	0.000	98	0.000	98	0.000	0	.	100	0.000	0.000
	53	197	0.000	0.000	296	0.000	199	0.000	295	0.002	300	0.000	0.000	295	0.000	296	0.002	300	0.000	99	0.000	197	0.000	0.000
	54	196	0.000	0.000	282	0.000	200	0.000	294	0.000	281	0.000	0.000	280	0.000	281	0.002	294	0.000	99	0.000	196	0.000	0.000
	55	83	0.000	0.000	83	0.000	83	0.000	83	0.000	83	0.000	0.000	83	0.000	83	0.000	83	0.000	83	0.006	83	0.000	0.000
	56	97	0.000	0.000	99	0.000	99	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	98	0.000	100	0.000	0.000
	57	100	0.000	0.000	236	0.000	100	0.000	243	0.000	213	0.000	0.000	243	0.012	243	0.000	236	0.000	100	0.000	100	0.000	0.000
	58	50	0.000	0.000	233	0.000	50	0.000	238	0.000	218	0.000	0.000	232	0.000	238	0.000	238	0.000	50	0.000	50	0.000	0.000
	59	0	.	.	154	0.000	0	.	154	0.000	154	0.000	0.000	144	0.000	154	0.000	154	0.000	0	.	0	.	.
	60	91	0.000	0.000	91	0.000	91	0.000	91	0.000	91	0.000	0.000	91	0.000	91	0.000	91	0.000	91	0.000	91	0.005	0.000
	61	100	0.000	0.000	98	0.000	100	0.000	97	0.000	99	0.000	0.000	99	0.000	100	0.000	100	0.000	86	0.000	99	0.000	0.000
	62	100	0.000	0.000	99	0.000	99	0.000	99	0.000	100	0.000	0.000	79	0.000	100	0.000	99	0.000	100	0.000	100	0.000	0.000
FRASER	63	99	0.000	0.000	97	0.000	99	0.000	99	0.000	98	0.000	0.000	99	0.000	99	0.000	96	0.000	0	.	98	0.000	0.000
	64	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000
	65	249	0.000	0.000	249	0.000	249	0.000	229	0.000	250	0.000	0.000	247	0.000	249	0.004	125	0.000	240	0.000	249	0.000	0.010
	66	99	0.000	0.000	99	0.000	99	0.000	99	0.000	99	0.000	0.000	99	0.000	99	0.000	99	0.000	99	0.000	99	0.000	0.000
	67	99	0.000	0.000	99	0.000	99	0.000	99	0.000	99	0.000	0.000	99	0.000	99	0.000	99	0.000	99	0.000	99	0.000	0.000
	68	98	0.000	0.000	99	0.000	100	0.000	98	0.000	96	0.000	0.000	99	0.000	100	0.000	100	0.000	95	0.000	100	0.000	0.000
	69	100	0.000	0.000	100	0.000	100	0.000	100	0.000	99	0.000	0.000	99	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000
	70	98	0.000	0.000	98	0.000	98	0.000	98	0.000	98	0.000	0.000	98	0.000	98	0.010	98	0.000	98	0.000	98	0.000	0.000
	71	42	0.000	0.000	42	0.000	42	0.000	42	0.000	42	0.000	0.000	42	0.000	42	0.000	42	0.000	42	0.000	42	0.000	0.000
	72	67	0.000	0.000	67	0.000	67	0.000	67	0.000	67	0.000	0.000	67	0.000	67	0.015	101	0.000	67	0.000	67	0.000	0.000
	73	101	0.000	0.000	101	0.000	101	0.000	101	0.000	101	0.000	0.000	101	0.000	101	0.080	130	0.000	101	0.000	101	0.000	0.000
	74	190	0.000	0.000	290	0.000	289	0.000	189	0.000	290	0.000	0.000	290	0.000	261	0.008	262	0.000	289	0.000	290	0.000	0.000
	83	90	0.000	0.000	90	0.000	90	0.000	90	0.000	90	0.000	0.000	90	0.000	90	0.011	90	0.000	90	0.000	90	0.000	0.000
	75	100	0.000	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.010	100	0.000	100	0.000	100	0.000	0.000
	76	100	0.000	0.000	101	0.000	101	0.000	242	0.000	100	0.000	0.000	101	0.000	100	0.000	101	0.000	242	0.000	100	0.000	0.000
	77	50	0.000	0.000	51	0.000	51	0.000	51	0.000	51	0.000	0.000	51	0.000	50	0.020	51	0.000	51	0.000	50	0.000	0.000
COLUMBIA	78	20	0.000	0.000	26	0.000	24	0.000	26	0.000	26	0.000	0.000	26	0.000	20	0.000	26	0.000	26	0.000	20	0.000	0.000
	79	102	0.049	0.000	102	0.000	102	0.000	102	0.000	102	0.000	0.005	102	0.000	101	0.015	102	0.000	102	0.000	102	0.000	0.000
	80	100	0.045	0.000	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.020	100	0.000	100	0.000	100	0.000	0.000

deviations at $p < 0.05$; again, 31 such deviations would be expected by chance alone at this critical level. None of the deviations were significant after allowing for multiple comparisons (at $p < 0.00008$) so replicate samples from each spawning site were pooled.

Genotype frequencies usually did not differ significantly among sites within the same nursery lake and were pooled to form "populations" for some subsequent analyses. However, spawning sites within Fred Wright Lake (Bonney Creek and Kwinageese River) and Shuswap Lake (Adams River and lower Shuswap River) were clearly distinct populations. Bonney Creek and Kwinageese River were consistently different ($p < 0.001$) at both *PGM-1** and *LDH-B2** in both years of sampling. Adams River and Shuswap River were significantly different ($p < 0.001$) at both *PGM-1** and *ALAT**, and these differences were consistent with those reported

TABLE 3D. Sample data for monomorphic loci ($q = 0$) and variant allele frequencies at two supplementary polymorphic loci. Sample from site 76 assayed at *mAAT-1** and *PEP-LT** by G. Winans and P. Aebersold, Northwest and Alaska Fisheries Center, Seattle, Wash. (personal communication).

River System	Site No.	cSPDH-B,4*		mIDHP-2*		LDH-A1*		LDH-A2*		mAAT-1*			PEP-LT*		
		(N)	*100	(N)	*100	(N)	*100	(N)	*100	(N)	*-80		(N)	*90	*110
ALSEK	1	76	1.000	76	1.000	76	1.000	76	1.000	75	0.013		76	0.000	0.000
TAKU	2	188	1.000	188	1.000	188	1.000	188	1.000	97	0.082		98	0.010	0.000
	3	269	1.000	269	1.000	270	1.000	270	1.000	100	0.160		100	0.000	0.000
	4	90	1.000	88	1.000	90	1.000	90	1.000	0	.		0	.	.
	5	175	1.000	175	1.000	175	1.000	175	1.000	0	.		0	.	.
STIKINE	6	300	1.000	300	1.000	304	1.000	304	1.000	0	.		0	.	.
	7	111	1.000	122	1.000	120	1.000	120	1.000	0	.		0	.	.
	8	210	1.000	211	1.000	213	1.000	213	1.000	0	.		0	.	.
	9	109	1.000	112	1.000	112	1.000	112	1.000	0	.		0	.	.
	10	161	1.000	167	1.000	166	1.000	166	1.000	0	.		0	.	.
	11	100	1.000	98	1.000	200	1.000	200	1.000	0	.		0	.	.
UNUK	12	0	.	49	1.000	49	1.000	49	1.000	0	.		0	.	.
NASS	13	168	1.000	168	1.000	168	1.000	168	1.000	0	.		0	.	.
	14	100	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	15	99	1.000	98	1.000	100	1.000	100	1.000	0	.		0	.	.
	16	251	1.000	256	1.000	262	1.000	262	1.000	0	.		0	.	.
	17	278	1.000	280	1.000	280	1.000	280	1.000	0	.		0	.	.
	18	192	1.000	199	1.000	200	1.000	200	1.000	0	.		0	.	.
	19	293	1.000	298	1.000	300	1.000	300	1.000	0	.		0	.	.
SKEENA	20	149	1.000	150	1.000	150	1.000	150	1.000	0	.		0	.	.
	21	80	1.000	81	1.000	81	1.000	81	1.000	0	.		0	.	.
	22	77	1.000	77	1.000	77	1.000	77	1.000	0	.		0	.	.
	23	162	1.000	162	1.000	162	1.000	162	1.000	0	.		0	.	.
	24	100	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	25	142	1.000	152	1.000	152	1.000	152	1.000	0	.		0	.	.
	81	93	1.000	93	1.000	93	1.000	93	1.000	93	0.140		93	0.000	0.000
	82	59	1.000	60	1.000	60	1.000	60	1.000	60	0.067		60	0.000	0.000
	26	75	1.000	75	1.000	74	1.000	74	1.000	0	.		0	.	.
	27	99	1.000	99	1.000	99	1.000	99	1.000	0	.		0	.	.
	28	98	1.000	98	1.000	98	1.000	98	1.000	0	.		0	.	.
	29	192	1.000	198	1.000	198	1.000	198	1.000	499	0.049		367	0.033	0.001
	30	178	1.000	179	1.000	179	1.000	179	1.000	0	.		0	.	.
	31	199	1.000	194	1.000	199	1.000	199	1.000	0	.		0	.	.
	32	164	1.000	164	1.000	164	1.000	164	1.000	0	.		0	.	.
	33	194	1.000	200	1.000	200	1.000	200	1.000	400	0.029		359	0.032	0.004
	34	78	1.000	78	1.000	78	1.000	78	1.000	0	.		0	.	.
	35	76	1.000	76	1.000	76	1.000	76	1.000	0	.		0	.	.
	36	160	1.000	164	1.000	164	1.000	164	1.000	0	.		0	.	.
	37	72	1.000	74	1.000	74	1.000	74	1.000	0	.		0	.	.
COASTAL	38	0	.	0	.	0	.	0	.	0	.		0	.	.
	39	0	.	0	.	0	.	0	.	0	.		0	.	.
	40	80	1.000	81	1.000	81	1.000	81	1.000	0	.		0	.	.
	41	49	1.000	49	1.000	49	1.000	49	1.000	0	.		0	.	.
	42	0	.	0	.	0	.	0	.	0	.		0	.	.
	43	92	1.000	90	1.000	92	1.000	92	1.000	0	.		0	.	.
	44	100	1.000	100	1.000	98	1.000	98	1.000	0	.		0	.	.
	45	99	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	46	100	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	47	45	1.000	45	1.000	45	1.000	45	1.000	0	.		0	.	.
	48	100	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	49	100	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	50	81	1.000	81	1.000	81	1.000	81	1.000	0	.		0	.	.
	51	100	1.000	100	1.000	98	1.000	98	1.000	0	.		0	.	.
	52	99	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	53	195	1.000	199	1.000	200	1.000	200	1.000	0	.		0	.	.
	54	194	1.000	200	1.000	196	1.000	196	1.000	0	.		0	.	.
	55	83	1.000	83	1.000	83	1.000	83	1.000	0	.		0	.	.
	56	99	1.000	99	1.000	99	1.000	99	1.000	0	.		0	.	.
	57	100	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	58	50	1.000	50	1.000	50	1.000	50	1.000	0	.		0	.	.
	59	0	.	0	.	0	.	0	.	0	.		0	.	.
	60	91	1.000	91	1.000	91	1.000	91	1.000	0	.		0	.	.
	61	97	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	62	96	1.000	99	1.000	100	1.000	100	1.000	0	.		0	.	.
FRASER	63	97	1.000	99	1.000	99	1.000	99	1.000	0	.		0	.	.
	64	100	1.000	100	1.000	100	1.000	100	1.000	100	0.645		100	0.000	0.000
	65	247	1.000	249	1.000	249	1.000	249	1.000	100	0.385		99	0.015	0.000
	66	99	1.000	99	1.000	99	1.000	99	1.000	99	0.010		99	0.131	0.000
	67	99	1.000	99	1.000	99	1.000	99	1.000	99	0.080		99	0.035	0.000
	68	94	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	69	100	1.000	100	1.000	100	1.000	100	1.000	99	0.177		100	0.115	0.000
	70	99	1.000	98	1.000	98	1.000	98	1.000	98	0.071		98	0.158	0.000
	71	42	1.000	42	1.000	42	1.000	42	1.000	42	0.095		29	0.136	0.000
	72	67	1.000	67	1.000	67	1.000	67	1.000	42	0.036		42	0.048	0.107
	73	101	1.000	101	1.000	101	1.000	101	1.000	50	0.000		50	0.180	0.040
	74	290	1.000	289	1.000	290	1.000	290	1.000	111	0.063		82	0.037	0.000
	83	90	1.000	90	1.000	90	1.000	90	1.000	90	0.161		60	0.100	0.000
	75	100	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.
	76	101	1.000	101	1.000	100	1.000	100	1.000	57	0.561		58	0.000	0.000
	77	51	1.000	51	1.000	51	1.000	51	1.000	0	.		0	.	.
COLUMBIA	78	26	1.000	24	1.000	26	1.000	26	1.000	0	.		0	.	.
	79	101	1.000	102	1.000	102	1.000	102	1.000	0	.		0	.	.
	80	100	1.000	100	1.000	100	1.000	100	1.000	0	.		0	.	.

by Utter et al. (1984) for previous collections. Significant heterogeneity was also detected at *ALAT** and *PEPC** within Lakelse Lake (Williams Creek vs. Scully Creek); at *LDH-B2** in Takla Lake (Dust Creek vs. others); and at *PGM-1**, *PGM-2**, and *ALAT** in Babine Lake (four unenhanced sites vs. enhanced Fulton River vs. enhanced Pinkut Creek). However, genotype frequency differences among sites in Lakelse, Babine, and Takla lakes may not reflect real differences among sites because differences were observed in only 1 yr (Lakelse and Takla), or else the pattern was not consistent

TABLE 4. Average level of polymorphism and mean heterozygosity within sockeye populations from different river systems calculated over 33 loci.

River system	No. of populations	No. of polymorphic loci			Mean heterozygosity
		mean	min.	max.	
Transboundary rivers[a]	8	7.6	5	10	0.049
Nass	6	6.8	6	8	0.039
Skeena	10	6.7	5	9	0.040
Coastal					
Queen Charlotte Is.	2	5.5	4	7	0.035
Vancouver Is.	5	6.6	4	10	0.037
North mainland	7	6.4	4	9	0.036
South mainland	5	5.8	4	9	0.036
All coastal lakes	19	6.2	4	10	0.036
Fraser	12	6.9	5	11	0.042
Columbia	2	6.5	5	8	0.052
All systems	57	6.7	4	11	0.041
Proportion polymorphic		0.203	0.121	0.333	—

[a]Includes the Alsek, Taku, Stikine and Unuk rivers.

TABLE 5. Number of polymorphic loci and variant alleles detected in sockeye by geographical region. All populations in Table 3 are included (except 1993 collections); number screened at all 33 loci shown in parentheses.

Region	River system	No. of populations	No. of loci polymorphic at		Variant alleles (excluding *100)	
			$q > 0$	$q > 0.05$	No.	% of total
Far north	Transboundary rivers	12 (8)	17	6	23	65.7
North	Nass	6 (6)	11	5	15	42.9
	Skeena	12 (10)	18	8	22	62.9
	Northern rivers	18 (16)	18	8	23	65.7
Coastal	Queen Charlotte Is.	5 (2)	9	5	10	28.6
	Vancouver Is.	7 (4)	13	5	14	40.0
	North mainland	7 (7)	11	7	14	40.0
	South mainland	6 (6)	11	4	19	54.3
	All coastal lakes	25 (18)	19	8	27	77.1
South	Fraser	13 (12)	13	8	19	54.3
	Columbia	2 (2)	10	5	14	40.0
	Southern rivers	15 (14)	14	9	22	62.9
All regions (Canada)		70 (57)	25	14	35	100.0

across samples where many comparisons were made (Babine). Even so, distinct sites within these lakes were treated as individual populations, resulting in a total of 57 populations screened at all 33 loci. An additional 12 populations were screened at fewer loci (Table 3), but were excluded from most analyses.

Variation Among Populations

Mean heterozygosity at 33 loci ranged from 2.3 to 5.6% (mean 4.1%) over the 57 populations (Table 3). No obvious difference in mean heterozygosity was evident among different river systems except that the typically small coastal populations were significantly less heterozygous than interior populations ($p < 0.005$, Wilcoxon–Mann–Whitney test; Table 4). Of the 33 nonselected loci, an average of 6.7 (20.3%) were polymorphic within the 57 individual populations (Table 4). Neither the average number of polymorphic loci per population nor the maximum number of polymorphic loci observed within river systems varied significantly among river systems ($p > 0.05$). Only 14–23 (40–65.7%) of the 35 variant alleles detected in Canada, were observed within any one interior river system; 27 variant alleles (77.1%) were found within the 25 coastal populations collectively (Table 5).

Geographical Structuring of Populations

Hierarchical gene diversity analysis of sockeye from 63 spawning sites screened at 33 loci indicated that 82.8% of total genetic variation occurred within spawning sites but only 1.0% occurred among spawning sites within nursery lakes (Table 6). The nursery lake was the most important level of differentiation among samples, accounting for 7.0% of variation within major drainages. Variation among drainages within river systems accounted for another 2.9 and 6.3%, respectively. The greatest structuring at the river system level occurred at *LDH-B2** (15.4%), *PGM-1** (10.8%), *ALAT** (5.2%), and *ADA-2** (3.8%).

TABLE 6. Distribution of electrophoretically detectable gene diversity among 63 sockeye collections screened at 33 loci.

Locus	Absolute gene diversity		Relative gene diversity				
	Total	Within sites	Within sites	Among sites within lakes	Among lakes within drainages	Among drainages within systems	Among river systems
Most variable loci							
*ALAT**	0.6180	0.5342	0.858	0.007	0.061	0.022	0.052
*PGM-1**	0.4116	0.2988	0.695	0.018	0.092	0.087	0.108
*PGM-2**	0.2420	0.2309	0.953	0.001	0.039	0.002	0.005
*LDH-B2**	0.1449	0.1066	0.715	0.031	0.117	−0.017	0.154
*PEPC**	0.0630	0.0590	0.936	0.002	0.052	0.016	−0.006
*ADA-2**	0.0348	0.0304	0.868	0.018	0.064	0.012	0.038
*GPI-B1,2**	0.0246	0.0223	0.906	0.002	0.102	−0.027	0.017
*AAT-3**	0.0184	0.0172	0.938	0.000	0.021	0.008	0.033
Averaged across all 33 loci			0.828	0.010	0.070	0.029	0.063

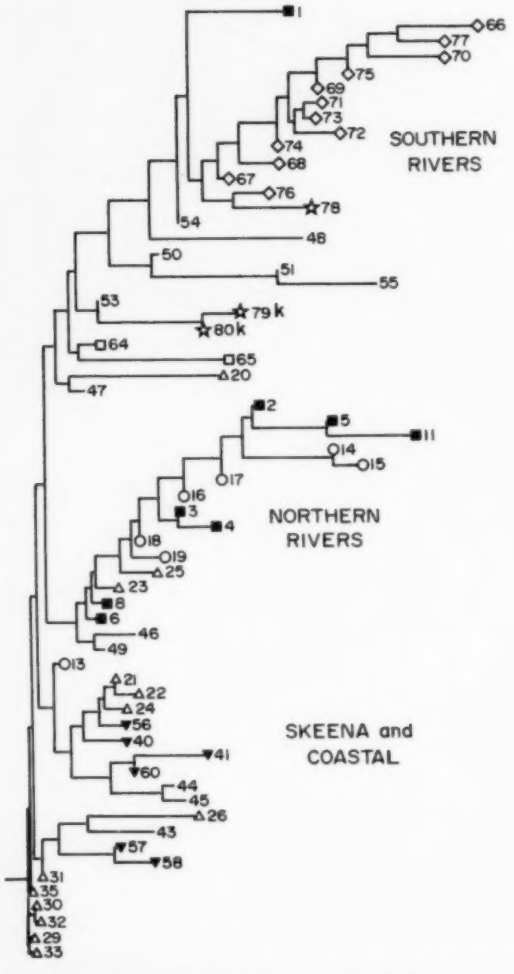

FIG. 2. Phylogenetic relationships among Canadian sockeye collections inferred from allozyme variation at 33 nonselected loci. Dendrogram constructed using neighbour-joining method (Saitou and Nei 1987) and Cavalli-Sforza and Edwards' (1967) chord distance. Numbers correspond to site numbers in Table 3; symbols correspond to river system or areas as in Fig. 4 except that symbols for coastal mainland sites have been omitted; *k* denotes kokanee.

Significant geographical structuring was also evident in the neighbour-joining dendrogram based on chord distance (Fig. 2). The large upper cluster included all samples from southern rivers (the Fraser and Columbia), whereas the large middle cluster included only samples from northern river systems (the Taku, Stikine, Nass, and Skeena; the Alsek was considered a northern river but the single site from that river clustered loosely with the southern rivers group). The small lower clusters included all coastal island sites, most coastal mainland sites, most Skeena River sites, and one site from the lower Nass River. Other coastal mainland sites, and the two sites from the lower Fraser (below Hell's Gate; see Table 1) clustered loosely with sites from the southern rivers. Thus, the geographical structuring was far from perfect and two populations in widely separated river systems sometimes resembled one another genetically more than they resembled other populations within their respective watersheds. The same "mosaic" pattern was evident in the UPGMA dendrogram based on Nei unbiased genetic distance (not shown). The UPGMA dendrogram often indicated very different branching patterns among individual populations, but it revealed the same distinct groupings of the northern rivers (excluding many Skeena populations), southern rivers, and Skeena–coastal populations.

Potential for Genetic Stock Identification

The 14 highly polymorphic loci screened in all 56 sockeye populations (kokanee excluded) were used to assess opportunities for genetic stock identification in mixed-stock fisheries by constructing a single-linkage similarity dendrogram from log-likelihood ratio distances that accumulate, rather than average, genotype frequency differences over multiple loci (Fig. 3). This procedure provides a quick way to compare the potential of different sets of genetic data for stock composition analysis with results obtained in simulation studies, and to test for statistical differences in genotype frequencies

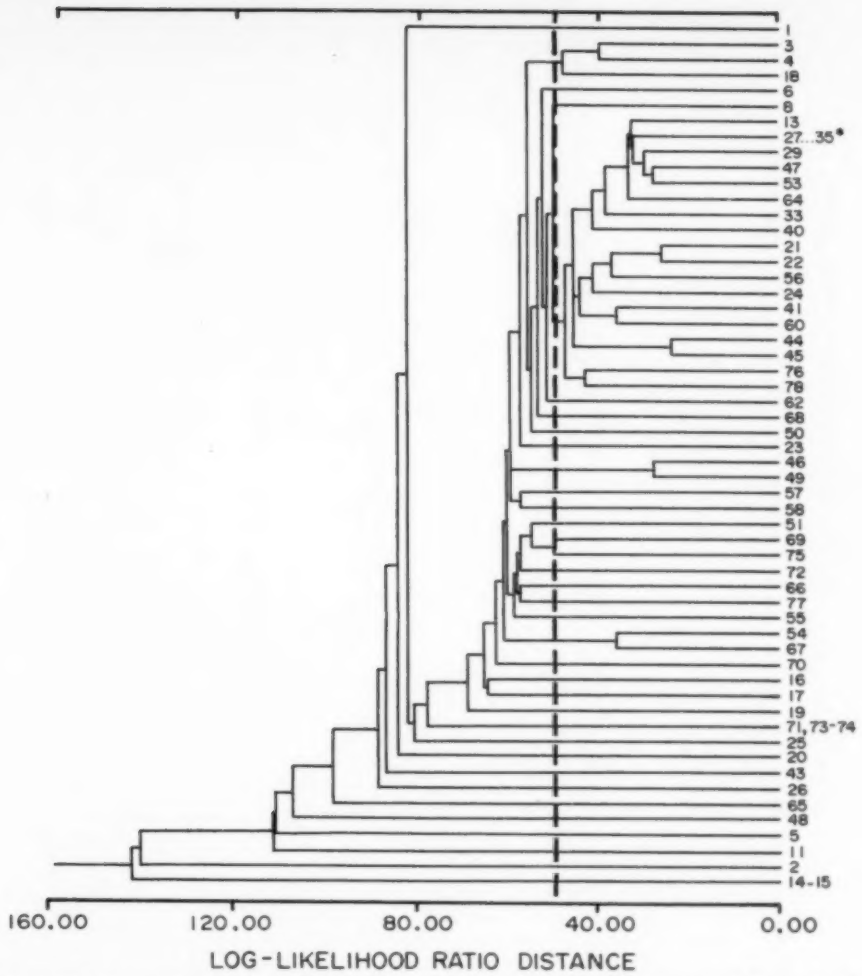

FIG. 3. Summary of potential for genetic stock identification of 56 Canadian anadromous sockeye populations using the 14 highly polymorphic loci in Table 3. The single linkage similarity dendrogram was constructed from Wood's (1989) log-likelihood ratio distances. Numbers correspond to site numbers in Table 3; some sites within lakes were pooled as indicated except that the grouping 27–35 does not include sites 29 and 33. See text for explanation of broken vertical line.

among populations (Wood 1989). The position of the broken vertical line in Fig. 3 has been inferred from previous simulation studies using fewer loci and subsets of the 57 populations (e.g., Wood et al. 1989). Although further simulations are required to establish the limits of stock composition analysis with the whole data set, we predict that populations that cluster to the right of the broken vertical line represent "problem clusters" that will be difficult or impossible to differentiate in mixed samples. On the other hand, populations that remain as distinct branches to the left of the broken vertical line are sufficiently unique that their individual contributions to mixed-stock fisheries can probably be estimated using maximum likelihood techniques (e.g., Fournier et al. 1984). It appears that many populations can be distinguished, but that some important populations from different river systems cannot be differentiated reliably using these 14 loci (e.g., Chilko (No. 67) and Long (No. 54) lakes, or Babine (Nos. 29–33), Harrison (No. 64), and Owikeno (No. 53) lakes). Unfortunately, this will probably limit our ability to estimate overall contributions from individual *river systems* in coast-wide mixed-stock fisheries.

Principal components analysis of allele frequency data at seven loci further illustrated why sockeye cannot always be distinguished reliably at the river system level (Fig. 4). The analysis was restricted to seven highly polymorphic loci to permit inclusion of additional "outgroup" samples from northern rivers (Kuril Lake, Iliamna Lake) and southern rivers (Arrow Lake kokanee). Principal components 1 and 2 (shown) account for only 44% of the total variation among populations, but they reveal most of the regional clustering; other components tend to separate individual populations. Upper Fraser and Columbia River populations clustered together as a "southern river" group that was distinct from populations in the remaining "northern river" systems. Coastal populations were plotted separately as "island" (Queen Charlotte and Vancouver islands) versus "mainland" groups. Lower Fraser River populations (those below the Fraser River canyon) were included in the coastal

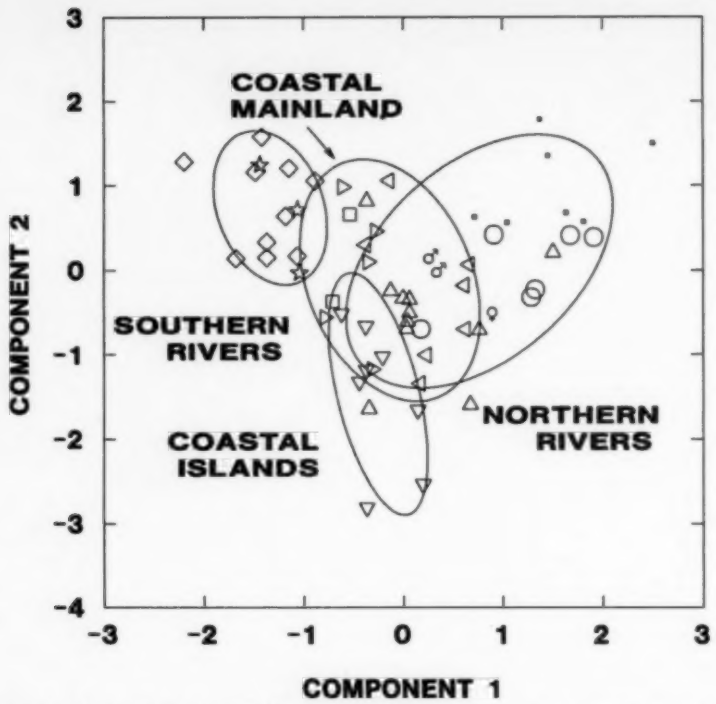

FIG. 4. Principal components analysis of arcsine-transformed allele frequencies at the seven most variable loci. Symbols denote major river systems or drainages as follows. (1) Northern rivers group: ■ Alsek, Taku, Stikine rivers; ○ Nass River; △ Skeena River; ♂ Iliamna Lake (Alaska); ♀ Kuril Lake (Kamchatka). (2) Southern rivers group: ◇ upper Fraser River and Thompson River; ☆ Columbia River. (3) Coastal islands group: ▼ Queen Charlotte Islands and Vancouver Island. (4) Coastal mainland group: ◁ north coast; ▷ central-south coast; □ lower Fraser River. Ellipses are 80% contours for normal bivariate distributions fitted to data for populations in each group. Components 1 and 2 accounted for 27 and 17% of the total variation, respectively.

mainland group because they were distinct from upper Fraser populations. Both coastal groups overlapped the northern rivers group at the extreme end of its distribution, nearest the southern rivers group, but the island group overlapped least. It is worth noting that similar patterns were evident independently in joint distributions of highly structured loci for which data are available for many more sites (e.g., *LDH-B2*115* vs. *PGM-1*-100* in 79 populations, Fig. 5A; *ADA-2*100* vs. *ALAT*91* in 66 populations, Fig. 5B). In all cases, reference samples included to represent populations derived from glacial refuges in Beringia (Kuril Lake and Iliamna Lake) and the Columbia River, appeared near the centre of the northern river and southern river clusters, respectively.

Discussion

The low value for mean heterozygosity (mean 4.1%, range 2.3–5.6%) observed in this study was similar to that for Cook Inlet sockeye screened at 26 loci (range 3.6–5.3% reported in Grant et al. 1980; 2.4–4.7% in Wilmot and Burger 1985). In studies where more (68+) loci have been examined, mean heterozygosity has been found to be slightly lower (range 2.1–4.1 in Utter et al. 1984; G. Winans, Northwest and Alaska Fisheries Center, Seattle, WA, personal communication). All these studies confirm that sockeye are typically less polymorphic than most other Pacific salmonids except perhaps coho salmon (*Oncorhynchus kisutch*) (Utter et al. 1973; Allendorf and Utter 1979; but see Milner 1993).

The nursery lake is clearly the primary unit of differentiation among sockeye populations in Canada, and in other regions (Grant et al. 1980; Utter et al. 1984; Wilmot and Burger 1985). This probably reflects the fact that sockeye home to their natal lake system with great fidelity, apparently to a greater degree than other Pacific salmon (Hanamura 1966; Quinn et al. 1987). Presumably, such fidelity is advantageous because it ensures that juvenile sockeye, which unlike other Pacific salmon are typically adapted for limnetic life, have access to a suitable rearing area. It may also be that sockeye simply make fewer errors homing to lakes because individual lakes have more distinctive odours than individual streams (Quinn 1985).

It is noteworthy that allozyme allele frequencies were not significantly different among collections of the river-rearing population(s) that spawn throughout vast areas of the Stikine River. In contrast, significant differences were observed among most lake-rearing populations, both within the Stikine River and other river systems (Table 3; more details in Wood et al. 1987b). These observations suggest that sufficient migration occurs between spawning sites within most nursery lakes, and perhaps within the river-rearing habitat of the Stikine River to prevent differentiation in selectively neutral allozymes from the effects of genetic drift and mutation. McCart (1970) reported that at least 12–25% of tagged fish displaced from spawning streams in Babine Lake strayed to other sites within the lake. Similar studies in other lakes have indicated comparable or

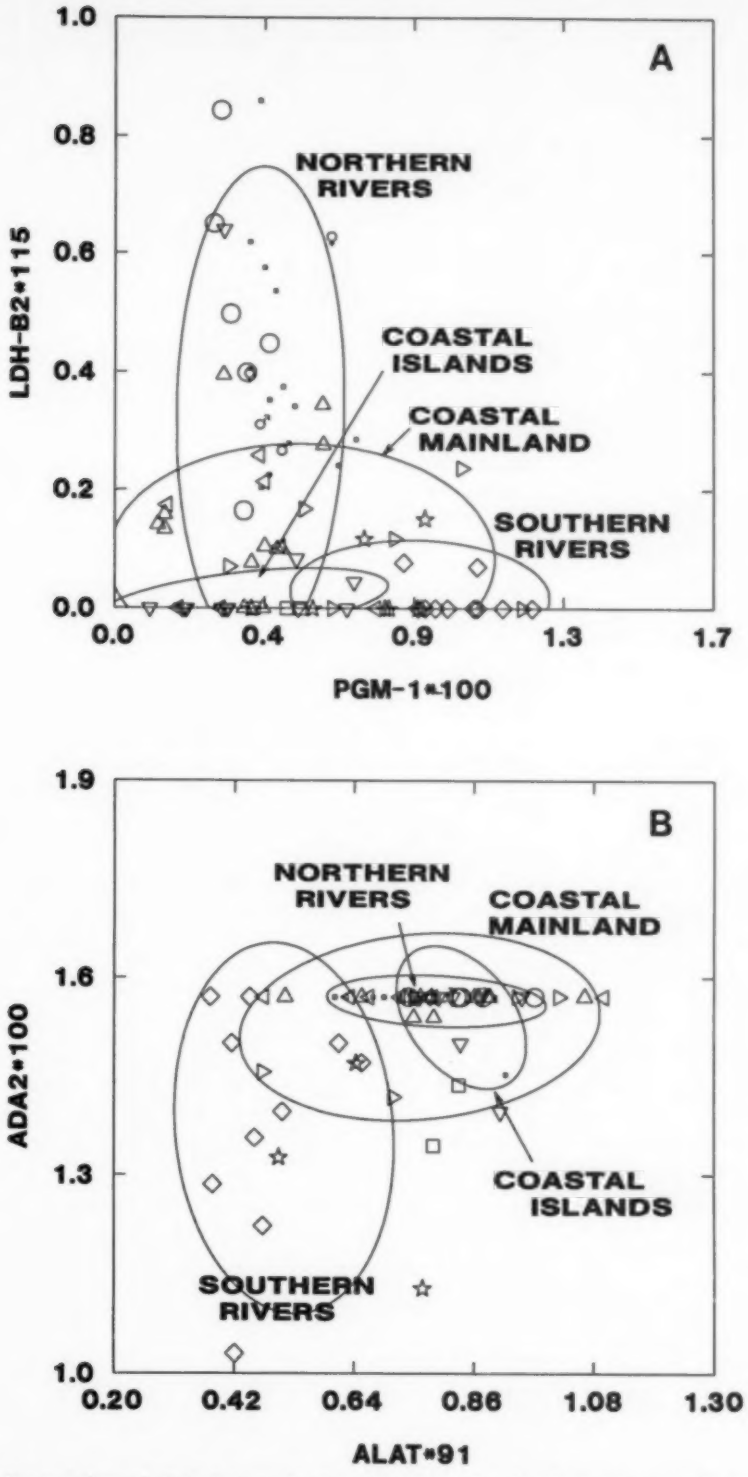

FIG. 5. Plots of joint arcsine-transformed allele frequencies for pairs of loci showing the most structuring at the river system level (Table 6). Symbols and groups are defined in Fig. 4. (A) *LDH-B2*115* versus *PGM-1*100* in 79 populations. Mercer Lake had an anomalous *LDH-B2*115* frequency and was excluded in computing ellipse for coastal islands group. (B) *ADA-2*100* versus *ALAT*91* in 66 populations. Transformed allele frequencies range from 0 (0%) to 1.57 radians (100%).

lower straying rates among tributaries (Hartman and Raleigh 1964; Varnavsky and Varnavskaya 1985). Even so, allozyme allele frequencies have sometimes revealed surprising differences among spawning sites within lakes (e.g., in Fred Wright and Shuswap lakes; also see Wilmot and Burger 1985; Varnavsky and Varnavskaya 1985; Foote et al. 1989; Varnavskaya et al. 1994b). Thus, we suspect that genetic differentiation among sites within lakes is actually very common with respect to traits such as spawning and migratory behaviour that are subject to strong natural selection (e.g., Averett and Espinosa 1968; Raleigh 1971; Wood and Foote 1990).

The remarkable differentiation of sockeye populations inhabiting different nursery lakes has afforded excellent opportunities for genetic stock identification techniques to be applied within river systems in British Columbia (e.g., Wood et al. 1987a; Rutherford et al. 1994) and in Cook Inlet, Alaska (Grant et al. 1980; Wilmot and Burger 1985; L. Seeb, Alaska Department of Fish and Game, Anchorage, AK, personal communication). It is ironic that this differentiation among lakes coupled with an insufficient number of highly polymorphic loci and rather limited differentiation on a regional scale, imposes severe limitations on genetic stock identification for coast-wide mixture problems. Adding the supplementary polymorphic loci listed in Table 3D will undoubtedly improve resolutions among some populations. Alternatively, other biological markers such as parasite "tags" can be included to provide greater regional structuring (e.g., Margolis 1963; Wood et al. 1989). Without strong regional structuring, it becomes necessary to characterize each population that might contribute significantly to a fishery.

Allozyme variation in sockeye from different nursery lakes appears to have been determined primarily by genetic drift during population bottlenecks associated with colonization events or fluctuations in abundance of small extant populations, where rare alleles have not been replenished by migration between neighbouring lakes. Several lines of evidence support this conclusion. First, differences between some neighbouring populations are very large (genetic distance > 0.01) considering that most if not all sockeye-producing lakes in Canada were covered by the Cordilleran Ice Sheet as recently as 10 000 – 15 000 yr BP (Clague et al. 1980). The magnitude of these differences is not consistent with rates of genetic drift expected in large isolated populations, but is consistent with rates expected for populations established from a small number of individuals (Nei 1987). Second, less than half of the total number of variant alleles observed in British Columbia were present in any single population. Coastal populations, which typically inhabit small relatively unproductive lakes, exhibited lower mean heterozygosity than interior populations. However, collectively, the coastal lakes exhibited as much, if not more, polymorphism than other river systems, indicating that selection was not responsible for allele distributions. Third, more of the variation in allele frequencies among populations occurs at the nursery-lake level than at the river-system level. In fact, it is common to find greater differentiation between two populations in the same drainage than between two populations from different river systems, or even on different continents (Varnavskaya et al. 1994a). This mosaic structuring is more easily explained by coincidental founder events than by phylogenetic descent or selection. Utter et al. (1984) reached similar conclusions regarding the anomalous allele frequencies of sockeye from Quinault Lake, a small coastal population in Washington.

Canadian sockeye are of particular interest because all extant populations were probably established within the last 10 000 – 15 000 years, subsequent to the late Wisconsin Glaciation. Sockeye are thought to have survived that glaciation in refuges in the Bering Sea region (Beringia) and the Columbia River (McPhail and Lindsey 1970; 1986; Lindsey and McPhail 1986). However, extremities of the Queen Charlotte Islands and Vancouver Island that were exposed by lowered sea levels during glaciation provided refuges for plants (Pojar 1980; Warner et al. 1982) and apparently some freshwater fishes (McPhail and Carveth 1992), and thus, perhaps sockeye. After examining geological evidence and the zoogeography of numerous freshwater species, McPhail and Lindsey (1970, 1986) concluded that many freshwater species from the Columbia refuge colonized the upper Fraser River when it flowed south through the Thompson River to the Okanagan Valley while ice dammed outflow to the coast through the Fraser River canyon. They also postulated a dispersal route from the present upper Fraser to the upper Skeena River through the upper Babine drainage while the lower Skeena River was still blocked by ice (McPhail and Lindsey 1986; Lindsey and McPhail 1986).

Geographical patterns of allele frequencies at the most geographically structured loci (*PGM-1**, *ALAT**, *LDH-B2**, and *ADA-2**) corroborate McPhail and Lindsey's (1970, 1986) general conclusions regarding post-glacial dispersal of sockeye. Northern rivers appear to have been colonized from the Bering refuge, and southern rivers from the Colombia refuge (Figs. 2–6, also Withler 1985). Note, however, that the coastal islands group is somewhat distinct from the southern rivers group and the northern rivers group (excluding some Skeena River sites) in both the neighbour-joining dendrogram (Fig. 2) and the principal components analysis (Fig. 4). This suggests that sockeye may have survived in ice-free coastal regions of the Queen Charlotte Islands and Vancouver Island. Also, the fact that coastal mainland and many Skeena River populations broadly overlap the other three groups suggests that they were founded by more than one ancestral type.

The discontinuity in allele frequencies at *PGM-1** and *ALAT** in the Fraser River canyon area is consistent with the geological and zoogeographic evidence that ice blocked this region, forcing water to flow from the upper Fraser into the Columbia through the Okanagan system. It is also remarkable that several populations in the postulated Fraser–Columbia and Fraser–Skeena transition zones share rare alleles that were not observed in other Fraser River populations. Within the Fraser River, variant alleles at *LDH-B2** and the rare *ALAT*108* allele occur only in the Dust Creek (Takla Lake) and Shuswap Lake populations; these are the populations located closest to the Babine (Skeena) and Okanagan (Columbia) drainages, respectively. The *LDH-B2*85* allele occurs nowhere in the Fraser River except Dust Creek (at a frequency of 6.7%), yet it occurs at all Babine Lake sites (1.3–4.3%) and is relatively common in some other Skeena populations (up to 12%). Similarly, the *ALAT*108* allele occurs only in Dust Creek (1.0%) and the Thompson River within the Fraser drainage, but occurs in many northern river systems including Babine Lake (0–1.9%). The *ALAT*108* allele occurs in the Thompson drainage in

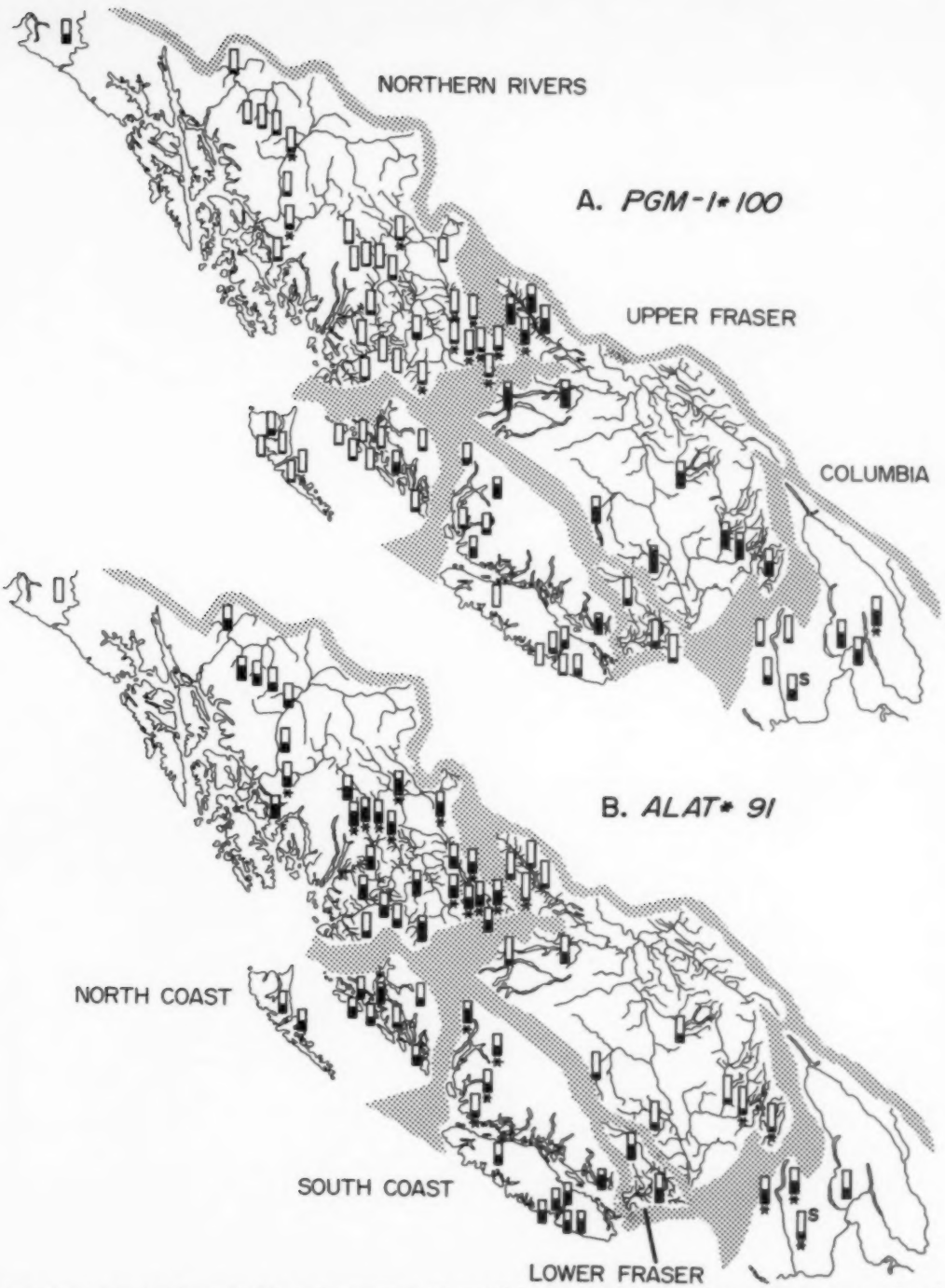

FIG. 6. Maps showing major discontinuities and occurrence of rare alleles in transition zones between areas apparently colonized by northern and southern ancestral races of sockeye. (A) *PGM-1*-100* allele frequencies (shaded part of bar chart) and occurrence of rare *LDH-B2*85* allele (asterisk). (B) *ALAT*91* allele frequencies (shaded part of bar chart) and occurrence of rare *ALAT*108* allele (asterisk). Columbia River populations are all kokanee except the Osoyoos Lake collection denoted *s*.

both Adams River (1.5%) and Lower Shuswap River (7.3%), but it also occurs at a higher frequency in the neighbouring Okanagan drainage (15.4% in Osoyoos Lake sockeye and 8.3–9.0% in Okanagan Lake kokanee). Both *ADA-2** and *GPI-B1,2** are highly polymorphic for the same variant alleles in some Columbia and Fraser river populations (including

the Shuswap and Adams lake populations), but monomorphic or less polymorphic in all other populations sampled. Slatkin (1985) has demonstrated that shared rare alleles provide a robust indication of the extent of gene flow between populations, or of past gene flow between populations that have been isolated for less than N generations where N is effective population size. Although the shared electromorphs are not necessarily identical alleles, it seems reasonable to infer that relatively recent gene flow has occurred between Takla and Babine lakes, and Shuswap and Okanaga lakes, given their geographical proximity and geological history.

All of the foregoing observations tend to corroborate McPhail and Lindsey's (1986) general conclusions regarding faunal exchange between the Thompson and Columbia rivers and between the upper Fraser and upper Babine rivers. However, McPhail and Lindsey (1986) concluded that fish penetrated the upper Skeena from the Fraser before the lower Skeena was free of ice; in contrast, the occurrence of rare "Skeena alleles" in Takla Lake, but not elsewhere in the Fraser, suggests that northern-type sockeye crossed the divide from the Skeena to the Fraser after the upper Skeena became accessible from the north coast, perhaps relatively recently. The biochemical evidence for recent exchanges of sockeye between Babine and Takla lakes is corroborated by additional evidence from the incomplete survey data for *PEP-LT** (Table 3D) and from two minisatellite DNA probes indicating that Takla Lake sockeye resemble Babine Lake sockeye more that Shuswap Lake (Taylor et al. 1995).

In summary, our results, and those from other recent studies of variation in sockeye allozymes (Utter et al. 1984; Withler 1985; Varnavskaya et al. 1994a), mitochondrial DNA (Bickham et al. 1995, and unpublished data) and minisatellite nuclear DNA (Beacham et al. 1995; Taylor et al. 1995) are consistent with the view that modern sockeye populations are derived from at least two ancestral types (a northern type from the Bering refuge and a southern type from the Columbia refuge) and probably a third type surviving on the outer coast of British Columbia. The northern type appears to have dispersed southward along the coast, penetrating inland up northern Canadian river systems and colonizing a number of coastal lakes and the lower Fraser River up to the Fraser River canyon. The southern type dispersed northward from the Columbia River, into the Thompson and upper Fraser rivers. Some exchange between the founding types also appears to have occurred in the vicinity of Babine and Takla lakes. Unfortunately, it was seldom possible to trace phylogenetic relationships among individual sockeye populations with any certainty owing to the influence of founder effects and population bottlenecks in populations so strongly isolated by homing instincts. Nevertheless, we believe that the general features of genetic variation among extant populations have helped to reveal large-scale patterns of post-glacial colonization.

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Genetic Variation in Sockeye Salmon (*Oncorhynchus nerka*) Populations of Asia and North America

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We investigated population structure in sockeye salmon, *Oncorhynchus nerka*, in relation to ecological characteristics and geographical distribution using allozyme allelic frequencies compiled from new and previously published analyses for 71 populations (300 collections) throughout the species' range. Up to 87 protein-coding loci were screened in some samples, but of these, 55% were monomorphic, and another 31% were only slightly polymorphic with variant allele frequencies <0.01. Only 8 loci were moderately or highly polymorphic with variant allele frequencies exceeding 0.10 in at least some collections. The highest variation ever reported in salmon was discovered at *LDH-B2** where the **100* allele was fixed in some populations but rare in others. Genetic differentiation was typically greater among populations within regions than among regions. However, stock composition analysis based on patterns of variation at the nine most useful loci appears to be feasible for most major stocks examined in this study. The low level of polymorphism in sockeye despite high variability at a few polymorphic loci is attributed to precise homing to natal rivers and interactions among several colonizing races that survived the Pleistocene ice age in isolated refugia with different habitat characteristics.

Nous avons examiné la structure des populations du saumon rouge, *Oncorhynchus nerka*, en relation avec l'écologie et l'aire de répartition en utilisant les fréquences alléliques des allozymes obtenues de nouvelles analyses et d'analyses déjà publiées de 71 populations (300 collections) échantillonnées dans toute l'aire de répartition de l'espèce. Jusqu'à 87 loci codeurs d'allozymes ont été identifiés dans certains échantillons, mais 55% de ceux-ci étaient monomorphes tandis qu'un autre 31% n'étaient que légèrement polymorphes, les fréquences alléliques variant moins de 0,01. Seuls 8 loci étaient modérément ou fortement polymorphes, les fréquences alléliques variantes étant supérieures à 0,10 dans au moins certaines collections. La plus forte variation signalée chez le saumon se situe à *LDH-B2**, où l'allèle **100* est fixe chez certaines populations, mais rares chez d'autres. La différenciation génétique était typiquement plus importante entre les populations d'une région qu'entre les régions. Par contre, il semble possible d'effectuer une analyse de la composition des stocks basée sur les régimes de variation aux neuf loci les plus utiles pour les principaux stocks étudiés dans le cadre de l'étude. Malgré la forte variabilité à quelques loci polymorphes, on attribue le faible niveau de polymorphisme chez le saumon rouge au retour précis vers les cours d'eau d'origine et aux interactions entre plusieurs races colonisatrices qui ont survécu à l'époque glaciaire du Pléistocène dans les refuges isolés où les habitats étaient différents.

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The sockeye salmon, *Oncorhynchus nerka*, occurs in most rivers along the Pacific coast of North America from northwestern Alaska to the Columbia River, but in Asia it is restricted to relatively small regions of the Kamchatka Peninsula and the northern coast of the Bering Sea (Fig. 1). Like other Pacific salmon (*Oncorhynchus spp.*), sockeye salmon typically spawn in clean gravel in shallow tributaries where surface water currents percolate through the redds. However, sockeye salmon are unusual in that some large populations utilize spawning habitat in lakes rather than rivers. These lake-spawning populations typically utilize underground springs to oxygenate their redds in the otherwise oxygen-poor substrate of lakes and deep rivers. Underground springs are rarely used by other Pacific salmon, presumably because springwater differs from surface water in its temperature regime, oxygen concentration, pH reaction, and other chemical features (Krokhin and Krogius 1937; Krokhin 1960). The sockeye salmon's ability to use both types of spawning habitat tends to complicate its population structure (Varnavskaya et al. 1994).

Sockeye salmon is one of the most valuable and abundant salmon species exploited by North American and Russian fisheries, and until recently by the Japanese high seas fishery. Effective methods for estimating stock

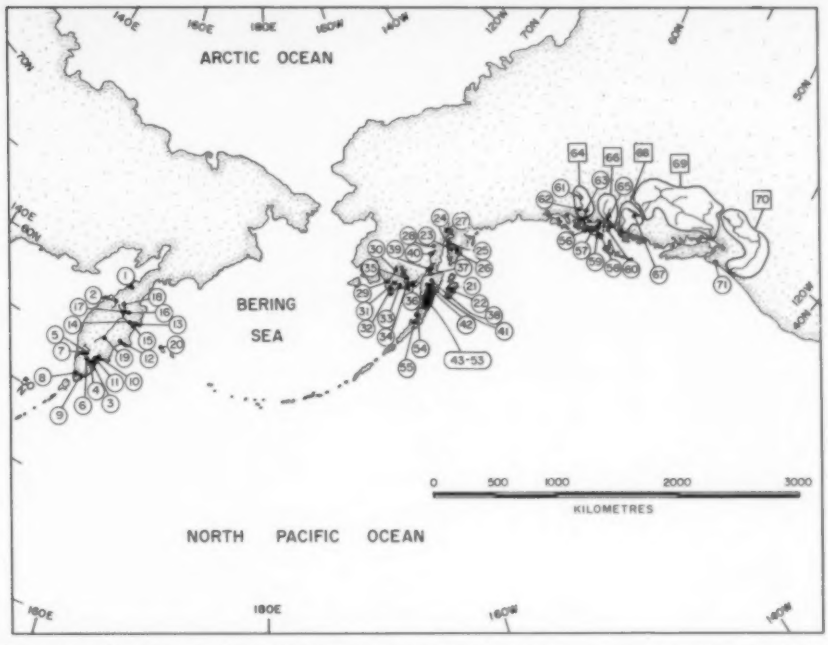

FIG. 1. Location of sample collections analyzed in the present study. Numbers correspond to those in Table 1. Numbers inside circles denote specific sites; those inside rectangles denote samples pooled within drainages.

composition of sockeye salmon catches in the Pacific Ocean are needed to successfully manage these fisheries. Some of the earliest research in fish population genetics was conducted on sockeye salmon (e.g., Hodgins et al. 1969; Utter and Hodgins 1970; Altukhov 1974; Altukhov et al. 1975), both because of interest in developing stock identification techniques and because of opportunities to investigate events in salmon evolution. These early investigators examined variation at two highly polymorphic loci coding the enzymes lactate dehydrogenase (*LDH-B2**) and phosphoglucomutase (*PGM-2**) discovered in sockeye tissues. Other loci were found to be less polymorphic in sockeye than in other salmonids, and after more then 10 yr of hard work the list of loci suitable for population genetic analysis was expanded to three highly polymorphic and three less polymorphic loci including those coding alanine aminotransferase (*ALAT**, previously *Gpt-4*), malate dehydrogenase (*MDH-B1,2**), glucosephosphate isomerase (*GPI**), and mannosephosphate isomerase (*MPI**) (Grant et al. 1981). A few years later, Utter et al. (1984) described two more highly polymorphic loci, *PGM-1** and *PEP-C** (previously *Gl-2*). These polymorphic loci were still insufficient for reliable stock composition analysis of mixed-stock fisheries, but they were useful for investigating the population structure of sockeye salmon (e.g., Altukhov et al. 1983; Muske 1983; Utter et al. 1984; Altukhov and Varnavskaya 1984; Varnavskaya 1984; Wilmot and Burger 1985; Withler 1985; Wood et al. 1987; Quinn et al. 1987; Varnavskaya et al. 1988; Foote et al. 1989; Wood et al. 1989, 1994).

Our purpose here is to document our recent findings from genetic studies of sockeye salmon in British Columbia, Alaska, and Kamchatka, and to compile previously reported data in a consistent format. We use these data to investigate sockeye population structure in relation to ecological characteristics and the geographical distribution of populations, and discuss the current feasibility of using biochemical genetic data to identify stock and continent of origin.

Methods

We compiled previously published allozyme allelic frequency data for 180 sample collections from Asian and North American sockeye salmon populations (Table 1). We also expanded this data set by performing electrophoretic analysis on 64 collections of sockeye salmon (total 4481 fish) from Kamchatka, 40 collections (total 2055 fish) from western Alaska, and 18 collections (total 1760 fish) from northern and central British Columbia, all collected between 1988 and 1991 (Table 1, Fig. 1). Tissue samples (skeletal muscle, heart muscle, liver, and eye retina) were collected from adult sockeye on their upstream migration in rivers or on their spawning grounds. Samples were usually frozen on dry ice or in liquid nitrogen.

Horizontal starch-gel electrophoresis was used to identify salmon genotypes at biochemical loci coding up to 38 different enzyme systems involved in metabolic activity in cytoplasm or on mitochondrial membranes. Six buffer solutions were used in electrophoresis as gel and electrode buffers (Table 2). Enzymes were revealed by incubation in substrate-containing stains following standard procedures (Aebersold et al. 1987). Genetic interpretation of electrophoretic patterns at most loci has been verified by comparing patterns in parents and their offspring. To ensure consistency of data collected by different agencies, many of the samples were analyzed cooperatively in laboratories at the Pacific Biological Station (Nanaimo, B.C.) and the Research Office of the U.S. Fish and Wildlife Service (Anchorage, AK). Electrophoretic mobilities of different

TABLE 1. Summary of allelic frequencies at 9 polymorphic loci used for genetic analysis, including sampling data and published sources.

Number, location and run timing[a]	Years	N[b]	Loci and alleles[c] *LDH-B2* *100	*PGM-1* *null	*PGM-2* *100	*GPI-B1,2* *100	*mAAT-1* *-100	*100	*ALAT* *91	*95	*MDH-B1,2* *100	*PEP-LT2* *100	*PEP-C* *100	Data source[d]
West Kamchatka														
1 Palana River	L 1979	1	0.872 (106)[e]	—	0.693 (106)	—	—	—	—	—	—	—	—	4
2 Hairusova River	L 1989	4	0.821 (336)	0.873 (336)	0.698 (336)	0.997 (321)	0.993 (335)	0.510	0.362 (334)	0.093	1.000 (335)	1.000 (313)	0.829 (178)	o.d.[f]
Bolshaya River System														
3 Nachiki Lake Basin	E 1980-82	35	0.681 (1337)	—	0.678 (1337)	—	—	—	—	—	—	—	—	7
4 Nachiki Lake	L 1979-83	6	0.930 (233)	—	0.715 (233)	—	—	—	—	—	—	—	—	7
5 Bistraya River	L 1989	3	0.842 (320)	0.962 (287)	0.689 (320)	0.992 (262)	0.981 (230)	0.506	0.380 (316)	0.109	1.000 (263)	1.000 (230)	0.923 (156)	o.d.
6 Plotnikova River	L 1978-79	2	0.757 (215)	—	0.691 (213)	—	—	—	—	—	—	—	—	4
7 Karimay Creek	L 1980	1	0.807 (42)	—	0.627 (42)	—	—	—	—	—	—	—	—	6
Ozernaya River System														
8 Ozernaya River	L 1984-87	5	0.829 (306)	—	0.651 (306)	—	—	—	—	—	—	—	—	o.d.
9 Kuril Lake and tributaries	L 1985-88	36	0.819 (2338)	0.827 (2310)	0.714 (2337)	1.000 (896)	—	0.511	0.396 (888)	0.048	1.000 (890)	—	—	o.d.
	L 1989	23	0.821 (1621)	0.836 (1339)	0.720 (1653)	0.998 (1459)	0.945 (984)	0.433	0.466 (1523)	0.065	0.990 (1495)	0.998 (1201)	—	o.d.
	L 1990	18	0.807 (876)	0.849 (858)	0.715 (894)	0.998 (835)	0.945 (867)	0.419	0.526 (813)	0.026	0.998 (815)	0.998 (815)	0.843 (585)	o.d.
East Kamchatka														
Paratunka River System														
10 Blignee Lake	L 1979-81	7	0.931 (160)	—	0.663 (160)	—	—	—	—	—	—	—	—	7
11 Dalnee Lake	L 1975	1	0.926 (66)	—	—	—	—	—	—	—	—	—	—	1
	L 1984-85	6	0.900 (297)	—	0.654 (295)	—	—	—	—	—	—	—	—	7
	L 1989-90	5	0.938 (260)	0.923 (257)	0.652 (258)	0.995 (260)	0.995 (99)	0.630	0.356 (250)	0.012	1.000 (260)	1.000 (255)	0.944 (94)	o.d.
Kronoki River System														
12 Kronoki Lake Basin	E 1970, 82	8	0.907 (508)	—	0.595 (560)	—	—	—	—	—	—	—	—	3,4
	L 1970, 82	3	0.765 (209)	—	0.591 (205)	—	—	—	—	—	—	—	—	3,4
Kamchatka River System														
13 Kamchatka River	E 1990-91	8	0.567 (347)	0.779 (339)	0.717 (330)	0.995 (347)	0.837 (339)	0.444	0.442 (341)	0.111	0.993 (346)	0.993 (339)	0.969 (334)	o.d.
14 Kamchatka River	L 1990-91	4	0.708 (268)	0.760 (267)	0.733 (267)	0.993 (268)	0.887 (263)	0.439	0.430 (254)	0.122	0.998 (264)	1.000 (264)	0.978 (257)	o.d.
15 Azabache Lake Basin	EL 1971-79	—	0.646 (5466)	—	0.785 (5466)	—	—	—	—	—	—	—	—	5

TABLE 1. (*Continued*)

Number, location and run timing[a]	Years	N^b	Loci and alleles[c]											Data source[d]
			LDH-B2 *100*	*PGM-1* *null*	*PGM-2* *100*	*GPI-B1,2* *100*	*mAAT-1* *-100*	**100*	*ALAT* *91*	**95*	*MDH-B1,2* *100*	*PEP-LT2* *100*	*PEP-C* *100*	
East Kamchatka (cont'd)														
16 Yelovka River	E 1987	1	0.680 (72)	—	0.732 (72)	—	—	—	—	—	—	—	—	o.d.
17 Dvu-Yurta River	L 1982	1	0.676 (34)	—	0.862 (34)	—	—	—	—	—	—	—	—	4
18 Dvu-Yurta Lake and tributaries	E 1982	3	0.184 (132)	—	0.808 (132)	—	—	—	—	—	—	—	—	7
19 Ushky Lake	L 1968	1	0.916 (101)	—	—	—	—	—	—	—	—	—	—	2
Kommandor's Islands														
20 Sarannoye Lake	L 1979	1	0.868 (72)	—	0.699 (72)	—	—	—	—	—	—	—	—	4
Kodiak Island														
21 Karluk Lake Basin	E 1978-81	6	0.904 (560)	—	0.758 (551)	—	—	—	—	—	—	—	—	10
22 Karluk Lake Basin	L 1978-81	5	0.846 (437)	—	0.758 (435)	—	—	—	—	—	—	—	—	10
Cook Inlet														
23 Kasiloff River Basin	L 1975-76	10	0.881 (879)	—	0.659 (879)	0.984 (879)	—	0.603 (879)	0.099 (879)	0.998 (879)	—	—	—	8
24 Kenai River Basin	L 1975-76	4	0.744 (420)	—	0.803 (420)	1.000 (420)	—	0.690 (420)	0.009 (420)	1.000 (420)	—	—	—	8
25 Russian River Basin	E 1978-81	4	0.519 (348)	—	0.886 (299)	—	—	—	—	—	—	—	—	10
26 Russian River Basin	L 1978-81	4	0.708 (353)	—	0.888 (249)	—	—	—	—	—	—	—	—	10
27 Susitna River	L 1975	4	0.952 (393)	—	0.553 (393)	1.000 (393)	—	0.575 (393)	0.037 (393)	1.000 (393)	—	—	—	8
Kuskokwim Bay Area														
28 Telaguana Lake	L 1989	1	0.680 (75)	0.529 (75)	0.873 (75)	1.000 (75)	0.780 (75)	0.813	0.140 (75)	0.047	1.000 (75)	1.000 (75)	—	o.d.
Kanektok River System														
29 Kanektok River	L 1989	1	0.910 (50)	0.927 (50)	0.700 (50)	1.000 (50)	0.920 (50)	0.270	0.530 (50)	0.180	1.000 (50)	1.000 (50)	—	o.d.
30 Kagaty Lake	L 1989	1	0.880 (50)	0.860 (50)	0.830 (50)	1.000 (50)	0.950 (50)	0.450	0.440 (50)	0.110	1.000 (50)	1.000 (50)	—	o.d.
Good News River System														
31 North Fork	L 1988	1	0.890 (50)	0.959 (50)	0.700 (50)	1.000 (50)	0.860 (50)	0.510	0.380 (50)	0.110	1.000 (50)	1.000 (50)	—	o.d.
32 Middle Fork	L 1988	1	0.870 (50)	0.883 (50)	0.730 (50)	1.000 (50)	0.890 (50)	0.480	0.330 (50)	0.190	1.000 (50)	1.000 (50)	—	o.d.

TABLE 1. (*Continued*)

Number, location and run timing[a]	Years	N^b	Loci and alleles[c]											
			LDH-B2 *100*	*PGM-1* *null*	*PGM-2* *100*	*GPI-B1,2* *100*	*mAAT-1* *-100*	**100*	*ALAT* *91*	**95*	*MDH-B1,2* *100*	*PEP-LT2* *100*	*PEP-C* *100*	Data source[d]
Bristol Bay Area														
33 Togiak River	L 1988	1	0.880 (50)	0.735 (50)	0.670 (50)	1.000 (50)	0.900 (50)	0.560	0.400 (50)	0.040	1.000 (50)	1.000 (50)	—	o.d.
34 Igushik River	L 1988	1	0.960 (50)	0.762 (50)	0.750 (50)	1.000 (50)	0.910 (50)	0.323	0.573 (48)	0.104	1.000 (50)	1.000 (50)	—	o.d.
35 Wood River	L 1988	1	0.970 (50)	0.883 (50)	0.760 (50)	1.000 (50)	0.750 (36)	0.370	0.580 (50)	0.050	1.000 (50)	1.000 (50)	—	o.d.
36 Nushagak River	L 1988	1	0.900 (50)	0.837 (50)	0.630 (50)	1.000 (50)	0.844 (45)	0.390	0.550 (50)	0.050	1.000 (50)	1.000 (50)	—	o.d.
Kvichak River System														
37 Battle Creek	L 1988	1	0.740 (50)	0.616 (50)	0.610 (50)	1.000 (50)	0.947 (47)	0.410	0.580 (50)	0.010	1.000 (50)	1.000 (50)	—	o.d.
38 Kulik Creek	L 1988	1	0.920 (50)	0.917 (50)	0.560 (50)	1.000 (50)	0.978 (46)	0.230	0.710 (50)	0.060	0.990 (50)	1.000 (50)	—	o.d.
39 Iliamma Lake Basin	L 1988-91	11	0.906 (564)	0.834 (560)	0.843 (559)	1.000 (560)	0.941 (539)	0.445	0.517 (553)	0.030	0.999 (555)	1.000 (554)	1.000 (155)	o.d.
40 Lake Clark Basin	L 1988	3	0.850 (150)	0.687 (150)	0.783 (150)	1.000 (150)	0.896 (150)	0.688	0.204 (149)	0.107	1.000 (150)	1.000 (150)	—	o.d.
Naknek River System														
41 Naknek Lake Basin	L 1984-85	5	0.789 (414)	—	0.729 (412)	—	—	—	—	—	—	—	—	11
	L 1989	1	0.900 (50)	0.917 (50)	0.660 (50)	1.000 (50)	0.890 (50)	0.380	0.520 (50)	0.100	1.000 (50)	1.000 (50)	—	o.d.
42 Brooks Lake Basin	L 1984-85	5	0.904 (310)	—	0.897 (310)	—	—	—	—	—	—	—	—	11
	L 1989	1	0.940 (50)	0.490 (50)	0.900 (50)	0.990 (50)	0.960 (50)	0.330	0.550 (50)	0.060	1.000 (50)	1.000 (50)	—	o.d.
Egegik River System														
43 Bible Creek	L 1988	1	0.910 (50)	0.860 (50)	0.790 (50)	1.000 (50)	0.937 (48)	0.360	0.470 (50)	0.170	1.000 (50)	1.000 (50)	—	o.d.
44 Kejulik River	L 1988	1	0.810 (50)	0.860 (50)	0.770 (50)	1.000 (50)	0.939 (50)	0.450	0.380 (50)	0.170	0.990 (50)	1.000 (50)	—	o.d.
45 Bear Creek	L 1988	1	0.900 (50)	0.872 (50)	0.730 (50)	1.000 (50)	0.880 (50)	0.316	0.480 (49)	0.204	1.000 (50)	1.000 (50)	—	o.d.
46 Ruth River	L 1988	1	0.930 (50)	0.833 (50)	0.600 (50)	0.990 (50)	0.936 (50)	0.286	0.531 (49)	0.184	1.000 (50)	1.000 (50)	—	o.d.
47 Franks Creek	L 1988	1	0.880 (50)	0.762 (50)	0.740 (50)	0.990 (50)	0.826 (50)	0.390	0.434 (50)	0.180	1.000 (50)	1.000 (50)	—	o.d.
48 Featherly Creek	L 1988	1	0.940 (50)	0.906 (50)	0.810 (50)	0.990 (50)	0.890 (50)	0.380	0.520 (50)	0.100	0.990 (50)	1.000 (50)	—	o.d.
Ugashik River System														
49 Ugashik River	L 1989	1	0.940 (50)	0.748 (50)	0.770 (50)	1.000 (50)	0.878 (50)	0.470	0.400 (50)	0.130	1.000 (50)	1.000 (50)	—	o.d.
50 Ugashik Narrows	L 1989	1	0.930 (50)	0.707 (50)	0.740 (50)	1.000 (50)	0.850 (49)	0.469	0.286 (49)	0.245	1.000 (50)	1.000 (50)	—	o.d.
51 Ugashik Creek	L 1989	1	0.950 (50)	0.775 (50)	0.720 (50)	1.000 (50)	0.927 (48)	0.550	0.340 (50)	0.110	1.000 (50)	1.000 (50)	—	o.d.
52 Deer Creek	L 1989	1	0.910 (50)	0.825 (50)	0.700 (50)	1.000 (50)	0.910 (50)	0.520	0.350 (50)	0.130	1.000 (50)	1.000 (50)	—	o.d.

TABLE 1. (*Continued*)

Number, location and run timing[a]	Years	N^b	Loci and alleles[c]											
			LDH-B2 *100*	*PGM-1* *null*	*PGM-2* *100*	*GPI-B1,2* *100*	*mAAT-1* *-100*	**100*	*ALAT* *91*	**95*	*MDH-B1,2* *100*	*PEP-LT2* *100*	*PEP-C* *100*	Data source[d]
Bristol Bay Area (cont'd)														
53 Black Creek	L 1989	1	0.920 (50)	0.812 (50)	0.670 (50)	1.000 (50)	0.889 (45)	0.460	0.400 (50)	0.140	1.000 (50)	1.000 (50)	—	o.d.
Port Moller														
54 Bear River	L 1989	1	0.940 (75)	0.887 (75)	0.893 (75)	1.000 (75)	0.840 (75)	0.460	0.473 (75)	0.067	1.000 (75)	1.000 (50)	—	o.d.
Nelson River System														
55 Sapsun Lake	L 1989	1	0.920 (50)	0.990 (50)	0.760 (50)	1.000 (50)	0.890 (50)	0.550	0.250 (50)	0.200	1.000 (50)	1.000 (50)	—	o.d.
Southeast Alaska														
56 Salmon Bay Creek	L 1982	1	0.993 (69)	0.768 (69)	0.841 (69)	1.000 (69)	—	0.200	0.323 (45)	0.478	1.000 —	1.000 —	0.888 (67)	9
57 Karta River	L 1982	1	0.950 (50)	0.800 (50)	0.760 (50)	1.000 (50)	—	0.716	0.011 (44)	0.273	1.000 —	1.000 —	0.970 (50)	9
58 Kegan Creek	L 1982	1	0.995 (100)	0.640 (100)	0.545 (100)	1.000 (100)	—	0.631	0.303 (99)	0.066	1.000 —	1.000 —	0.969 (98)	9
59 McDonald Lake	L 1982	1	0.980 (50)	0.920 (50)	0.860 (50)	1.000 (50)	—	0.711	0.244 (45)	0.045	1.000 —	1.000 —	0.920 (50)	9
60 Hugh Smith Lake	L 1982	1	0.910 (50)	0.810 (50)	0.850 (50)	1.000 (50)	—	0.580	0.352 (44)	0.035	1.000	1.000	0.925 (50)	9
British Columbia														
Stikine River System														
61 Tahltan Lake	E 1982-85	3	0.425 (400)	0.839 (384)	0.952 (397)	1.000 (393)	—	0.237	0.323 (99)	0.439	1.000 (299)	—	0.939 (74)	o.d.
62 Chutine Lake	L 1984-85	2	0.947 (150)	0.657 (148)	0.845 (148)	1.000 (148)	—	—	—	—	1.000 (150)	—	—	12
63 Christina Lake	L 1984-85	2	0.952 (121)	0.813 (75)	0.727 (75)	1.000 (118)	—	—	—	—	1.000 (122)	—	—	12
64 Stikine River and tributaries	L 1984-87	8	0.897 (939)	0.812 (927)	0.811 (875)	1.000 (614)	—	—	—	—	0.995 (715)	—	—	12
Nass River System														
65 Meziadin Lake Basin	E 1982-86	6	0.424 (591)	0.878 (585)	0.958 (592)	1.000 (585)	—	0.347	0.602 (384)	0.028	1.000 (592)	—	0.959 (394)	o.d.
66 Nass River Basin	E 1986-89	17	0.807 (1206)	0.875 (906)	0.863 (1199)	0.998 (1177)	—	0.252	0.616 (1087)	0.161	1.000 (1200)	—	0.951 (676)	o.d.
Skeena River System														
67 Babine Lake Basin	L 1982-90	10	0.968 (3225)	0.811 (3128)	0.770 (3207)	1.000 (2087)	0.960 (899)	0.421	0.490 (1979)	0.066	1.000 (1511)	0.938 (726)	0.920 (505)	o.d.
68 Skeena River	L 1987-88	8	0.962 (1170)	0.907 (1108)	0.767 (1109)	1.000 (1114)	—	0.402	0.494 (815)	0.090	1.000 (1169)	—	0.941 (496)	o.d.
Fraser River System														
69 Fraser River System	L 1982	6	0.998 (572)	0.565 (569)	0.825 (567)	0.945 (570)	—	0.572	0.303 (541)	0.081	1.000 —	1.000 —	1.000 (562)	9

TABLE 1. (Concluded)

Number, location and run timing[a]		Years	N[b]	Loci and alleles[c]											Data source[d]
				LDH-B2 *100*	*PGM-1* *null*	*PGM-2* *100*	*GPI-B1,2* *100*	*mAAT-1* *.100*	**100*	*ALAT* *91*	**95*	*MDH-B1,2* *100*	*PEP-LT2* *100*	*PEP-C* *100*	
Washington, U.S.A.															
70 Columbia River System	L	1982, 83	5	0.998 (556)	0.488 (565)	0.840 (534)	0.999 (205)	0.471 (69)	0.602	0.267 (378)	0.050	0.956 (90)	0.993 (70)	1.000 (323)	9,13,14
71 Quinault	L	1982	1	1.000 (50)	0.000 (50)	0.960 (50)	1.000 (50)	—	0.690	0.270 (50)	0.000	1.000 —	1.000 —	0.740 (50)	9

[a]Run timing: E, early sockeye, entering rivers in June and spawning in July to August; L, late sockeye, entering rivers in July to September and spawning in August to November, sometimes until December to January.
[b]N is the number of samples collected from each location.
[c]Rare alleles pooled for all loci except *ALAT** where only the rare alleles *86 and *108 were pooled.
[d]Literature sources cited: Printed in Russian: 1, Kirpichnikov and Ivanova 1977; 2, Chernenko et al. 1980; 3, Kirpichnikov and Muske 1981; 4, Muske 1983; 5, Altukhov et al. 1983; 6, Varnavskaya 1984; 7, Varnavskaya et al. 1988. Printed in English: 8, Grant et al. 1981; 9, Utter et al. 1984; 10, Wilmot and Burger 1985; 11, Wilmot et al. 1986; 12, Wood et al. 1987; 13, Foote et al. 1989; Wood et al. 1994.
[e]Number of fish successfully scored at each locus is in parentheses.
[f]o.d. indicates previously unpublished data.

alleles and loci of sockeye samples from Canada, Russia, and the United States were scored simultaneously on the same gels to verify that comparable results were obtained for subsequent statistical analyses.

Statistics describing genetic variation were calculated using BIOSYS-1 (Swofford and Selander 1981). Genetic variances within populations and regions were compared using Bartlett's test (Systat Inc. 1988). Heterogeneity of allelic frequencies among samples was examined by log-likelihood ratio analysis (G test, Sokal and Rohlf 1981). G tests were used first to examine heterogeneity among five geographical regions (western Kamchatka, eastern Kamchatka, western Alaska, southeastern Alaska, and British Columbia), and second to compare allelic frequencies in replicate collections within 11 major drainages ("stocks") sampled at least three times and at more than six loci. The 11 stocks included Kuril Lake, Kamchatka River, Egegik River, Ugashik River, Lake Clark, Iliamna Lake, Meziadin Lake, Babine Lake, Fraser River, and Columbia River.

Principal components analyses and neighbor-joining trees were used to examine genetic relationships among samples, stocks, and geographical regions. Principal components were calculated (using Systat Inc. 1988) from arcsine-transformed allelic frequencies at eight loci in eight stocks. Neighbor-joining trees (Saitou and Nei 1987) were constructed using allelic frequencies at eight loci to calculate pairwise chord distances (Cavalli-Sforza and Edwards 1967) among collection sites (replicate samples pooled).

Results

New and previously published data on principal allelic frequencies at nine polymorphic loci selected for subsequent analysis are summarized in Table 1. Although electrophoretic analysis of 37 enzyme systems allowed us to detect and score variation at up to 87 loci in some (but not all) samples, most loci (48) were monomorphic. Another 27 loci were polymorphic with variant alleles occurring at a very low frequency (<0.01) in a few collections but absent in most (Table 2). Only eight loci (*LDH-B2**, *mAAT-1**, *ALAT**, *PEP-C**, *mAH-1,2**, *PGM-1**, *PGM-2**, and *PGM-3,4**) were polymorphic with variant alleles occurring at a moderate to high frequency (>0.10) in at least some samples. Another five loci (*MDH-B1,2**, *PEP-LT2**, *mAH-3**, *mAH-4**, and *GPI-B1,2**) were less variable (0.01–0.10) but should be useful for genetic analysis. Of the 13 most polymorphic loci, only nine were included in statistical analyses of population structure. Interpretation of variation at *PGM-3,4** was considered unreliable for genetic analysis because too many alleles were detected, and their biochemical activity was unstable. Variation at the three mitochondrial aconitate hydratase loci (*mAH-1,2**, *mAH-3**, and *mAH-4**) has not been reported previously and consequently, data were not available for many populations.

The highest variation ever reported among salmon populations was discovered at *LDH-B2** with frequencies of *LDH-B2*100* ranging from 0.182 in Dvu-Yurta Lake in the Kamchatka River to 1.000 in the Fraser and Columbia rivers (Tables 1 and 3). In other words, this allele has become fixed in some populations but remains rare in others. Large variations in allelic frequencies among populations were also observed at *PGM-1**, *PGM-2** and *ALAT** (Table 3, Fig. 2). Genetic variances at *LDH-B2**, *PGM-1**, *ALAT**

TABLE 2. Enzyme systems, loci and alleles analyzed in some sockeye salmon populations of Asia and North America in 1988-1991.

Enzyme	IUBNC number	Locus	Tissue[a]	Allele mobilities					Gel buffer[b]	Level of variation[c]
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	M	100	150				6	$q \leq 0.010$
		*G3PDH-2**	H	100					6	Invariant
		*G3PDH-3**	H	100					6	Invariant
Lactate dehydrogenase	1.1.1.27	*LDH-A1**	M	100	105?				2, 3	$q \leq 0.010$
		*LDH-A2**	M	100	105				2, 3	$q \leq 0.010$
		*LDH-B1**	M, H, E	100	123				2, 3	$q \leq 0.010$
		*LDH-B2**	M, L, E	100	115	85			2, 3	$q \geq 0.100$
		*LDH-C**	E	100	109	92			2, 3	$q \leq 0.010$
Malate dehydrogenase NAD	1.1.1.37	*MDH-A1**	L, H, E	100	64	146			4	$q \leq 0.010$
		*MDH-A2**	L, H, E	100					4	Invariant
		*MDH-B1,2**	M, H, E	100	65	120	60		4	$0.010 \leq q \leq 0.100$
		*mMDH-1**	H	100	153				6	$q \leq 0.010$
		*mMDH-2**	H	100					6	Invariant
Malate dehydrogenase NADP (Malic enzyme)	1.1.1.40	*mMEP-1**	M, H	100	109				1	$q \leq 0.010$
		*mMEP-2**	M, H, L, E	100					1	Invariant
Isocitrate dehydrogenase	1.1.1.42	*mIDHP-1**	M, H	100					4	Invariant
		*mIDHP-2**	M, H	100	64				4	$q \leq 0.010$
		*IDHP-1**	E	100	123	84			4	$q \leq 0.010$
		*IDHP-2**	E	100	162	72			4	$q \leq 0.010$
6-Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M, H	100					4	Invariant
Glyceraldehyde-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	M	100					4	Invariant
		*GAPDH-2**	H	100					6	Invariant
		*GAPDH-3**	H	100					6	Invariant
		*GAPDH-4**	E	100	107				4	$q \leq 0.010$
		*GAPDH-5**	E	100					4	Invariant
Xanthyne oxidase	1.2.3.2	*XO**	L	100					2	Invariant
Glutathion reductase	1.6.4.2	*GR**	E	100					3	Invariant
Superoxide dismutase	1.15.1.1	*mSOD**	H	100					2	Invariant
		*SOD-1**	L	100					2	Invariant
		*SOD-2**	L	100					2	Invariant
Purine nucleoside phosphorilase	2.4.2.1	*PNP-1**	E	100					4	Invariant
		*PNP-2**	E	100					3, 4	Invariant
Aspartate aminotransferase	2.6.1.1	*AAT-1,2**	M, H	100	77	122			4	$q \leq 0.010$
		*AAT-3**	E	100	117				2, 3	$q \leq 0.010$
		*AAT-4**	L	100					4	Invariant
		*mAAT-1**	M, H	−100	−82				4	$q \geq 0.100$
		*mAAT-2**	L	−100	−64				4	$q \leq 0.010$
Alanine aminotransferase	2.6.1.2	*ALAT**	M, H	100	91	95	86	108	5	$q \geq 0.100$
Pyruvate kinase	2.7.1.40	*PK-1**	H	100					6	Invariant
		*PK-2**	M, H, E	100	105				4	$q \leq 0.010$
Phosphoglycerate kinase	2.7.2.3	*PGK-2**	M, H, E	100	−57				4	$q \leq 0.010$
		*PGK-2**	M, H, E	100					4	Invariant

TABLE 2. (*Continued*)

Enzyme	IUBNC number	Locus	Tissue[a]	Allele mobilities				Gel buffer[b]	Level of variation[c]
Creatine kinase	2.7.3.2	*CK-A1**	M	100	113			3	$q \leq 0.010$
		*CK-A2**	M	100				3	Invariant
		*CK-C1**	E	100				3	Invariant
		*CK-C2**	E	100				3	Invariant
		*CK-B**	E	100	102			3	$q \leq 0.010$
Adenylate kinase	2.7.4.7	*AK**	E	100				4	Invariant
Esterase D	3.1.1..	*EST-D**	M	100				3	Invariant
Hydroxyacylglutathione hydrolase	3.1.2.6	*HAGH**	M, H	100				4	Invariant
Acid phosphatase	3.1.3.2	*ACP-1**	L	100				2	Invariant
		*ACP-2**	L	100				2	Invariant
α-glucosidase	3.2.1.20	*α-GLU**	L					model uncertain	
N-Acetyl-β-glucosaminidase	3.2.1.31	*β-GLUA**	M	100				3	Invariant
β-Acetylgalactose aminidase	3.2.1.53	*β-GALA**	L	100				1	Invariant
Peptidases	3.4.....								
Leucyl-glycyl-glycine(LGG,TAPEP)		*PEP-B1**	M, H, E	100				2	Invariant
		*PEP-B2**	M, H, E	100				2	Invariant
Glycyl-leucine(GL, DPEP)		*PEP-A**	M, H, L, E	100	106			2	$q \leq 0.010$
		*PEP-C**	E	100	105			2	$q \geq 0.100$
Leucyl-tyrosine		*PEP-LT1**	M, H	100				2	Invariant
		*PEP-LT2**	M, H	100	92	114		2	$0.010 \leq q \leq 0.100$
Phenylalanine-proline(PP,PDPEP)		*PEP-D1**	H, E	100				2, 4	Invariant
		*PEP-D2**	H, E	100	105			2, 4	$q \leq 0.010$
Leucyl-glycine-leucine		*LGL**	M	100				3	Invariant
Guanine deaminase	3.5.4.3	*GDA-1**	L	100				1	Invariant
		*GDA-2**	L	100				1	Invariant
Adenosine deaminase	3.5.4.4	*ADA-1**	M	100	107			3	$q \leq 0.010$
		*ADA-2**	M	100	95			3	$q \leq 0.010$
Nucleosidetriphosphate pyrophosphatase	3.6.1.19	*NTP**	M	100				3	Invariant
Fumarate hydratase	4.2.1.2	*FH**	M	100	73			4	$q \leq 0.010$
Aconitate hydratase	4.2.1.2	*AH-1**	M, C	100	110?			4	Invariant
		*AH-2**	M, H, L	100				4	Invariant
		*AH-3**	M, H, L	100	117			4	$q \leq 0.010$
		*mAH-1,2**	M, H	100	87	110	65	4	$q \geq 0.100$
		*mAH-3**	M, H	100	85			4	$q \leq 0.010$
		*mAH-4**	M, H	100	126			4	$0.010 \leq q \leq 0.100$
Fructose-1,6-biphosphate aldolase	4.1.2.13	*FBALD-3**	E	100				4	Invariant
		*FBALD-4**	E	100	87			4	$q \leq 0.010$
Triosephosphate isomerase	5.3.1.1	*TPI-1**	H, E	100				2, 3	Invariant
		*TPI-2**	H, E	100				2, 3	Invariant
		*TPI-3**	H, E	100	110			2, 3	$q \leq 0.010$
		*TPI-4**	H, E	100				2, 3	Invariant

and *PEP-C** were significantly greater among stocks than within stocks ($P < 0.001$, Bartlett's test) (Table 3).

When all polymorphic loci were considered simultaneously, highly significant heterogeneity in allelic frequencies among samples was detected within all five regions examined ($P < 0.001$, Table 4). Significant heterogeneity was also detected within all stocks examined except Bistraya River, Ugashik River and Lake Clark.

TABLE 2. (*Concluded*)

Enzyme	IUBNC number	Locus	Tissue[a]	Allele mobilities				Gel buffer[b]	Level of variation[c]
Mannosephosphate isomerase	5.3.1.8	*MPI**	M, H	100	107			2	$q \leq 0.010$
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A**	M	100	94	107	86	3	$q \leq 0.010$
		*GPI-B1,2**	M	100	132	143		3	$0.010 \leq q \leq 0.100$
Phosphoglucomutase	5.4.2.2	*PGM-1**	M, H, E	−100	0			4	$q \geq 0.100$
		*PGM-2**	M, H, E	−100	−77	−93	−125	4	$q \geq 0.100$
		*PGM-3,4**	M, H	100	105	110	115	2	$q \geq 0.100$

[a]Tissue: M = muscle; H = heart muscle; E = eye retina; L = liver.
[b]Buffer systems (Aebersold et al. 1987): 1 = Tris-citrate buffer, pH 5.8 (TC-4); 2 = Tris–EDTA–boric buffer, pH 8.5; 3 = Ridgway buffer (TBCL), pH 8.0; 4 = Aminopropylmorpholine–EDTA–citrate buffer, pH 6.1 (ACE); 5 = Tris–glycine buffer, pH 8.9 (0.04 M Tris, 0.12 M glycine, equal concentration for gel and electrode); 6 = Aminopropylmorpholine–citrate buffer, pH 7.0 (AC).
[c]Level of variation: q designates the maximum frequency of rare alleles in any single sample.

TABLE 3. Summary of genetic variability at the nine most polymorphic loci in populations of sockeye salmon from Asia and North America. All collections listed in Table 1 were used to calculate relative frequencies of common alleles ($\bar{q}_{min}, \bar{q}_{max}, \bar{q}_{aver}$) and expected average heterozygosity (H_{exp}); however, only data from 10 stocks for which more than three collections from different sites were available, and where variation was detected in all collections were used in the analyses of genetic variance.

Locus	$\bar{q}_{min}$	$\bar{q}_{max}$	$\bar{q}_{aver}$	H_{exp}	Genetic variation: Among	Genetic variation: Within	Bartlett's test: (df = 9)	Bartlett's test: P
*LDH-B2**	0.182	1.000	0.836	0.274	0.0216	0.0011	103.8	<0.001
*PGM-1**	0.346	1.000	0.749	0.375	0.0076	0.0039	36.3	<0.001
*PGM-2**	0.545	0.975	0.808	0.310	0.0150	0.0021	22.6	<0.05
*ALAT**	0.230	0.813	0.483	0.499	0.0143	0.0054	29.7	<0.01
*mAAT-1**	0.825	1.000	0.942	0.109	0.0017	0.0013	25.5	<0.05
*PEP-C**	0.796	1.000	0.944	0.106	0.0042	0.0019		
*GPI-B1,2**	0.777	1.000	0.993	0.014	0.0010	0.0000		
*MDH-B1,2**	0.980	1.000	0.999	0.004	0.0000	0.0000		
*PEP-LT2**	0.948	1.000	0.999	0.004	0.0001	0.0000		

The first neighbor-joining tree (Fig. 3) was constructed from pairwise chord distances between 33 stocks listed in Table 1 using allelic frequencies at seven polymorphic loci (excluding *mAAT-1** and *PEP-C**); allelic frequencies from different collections within each stock were pooled for this analysis but in some cases we had to rely on data from a single collection. All stocks from the Alaska Peninsula, Kuskokwim Bay, and Bristol Bay (except Iliamna Lake), clustered together to form a western Alaska grouping although each stock was genetically distinct. The Iliamna Lake stock did not fall within the western Alaska grouping, and instead, clustered tightly with stocks from western Kamchatka, eastern Kamchatka, and northern British Columbia. Stocks from southeastern Alaska, the Fraser River, and Washington State were well separated from stocks in other regions and from each other, but did not form tight regional clusters.

The second neighbor-joining tree (Fig. 4) was constructed from pairwise chord distances between all collections from nine stocks for which allelic frequency data were available at eight polymorphic loci (excluding *PEP-C**). This tree shows the genetic relatedness of stocks more accurately than Figure 3. Again, three distinct clusters are evident: (1) the western Alaska group (including Egegik and Ugashik rivers and Lake Clark), (2) the Columbia–Fraser group, and (3) the Kamchatka–northern British Columbia group (including Kuril, Meziadin, Babine, and Iliamna lakes). Collections from the Columbia, Fraser, Ugashik and Egegik, and Lake Clark stocks displayed considerable heterogeneity, as expected since these stocks comprised different lakes within each watershed. Collections from sites within a single lake system (Kuril, Meziadin, Babine and Iliamna stocks) were more homogeneous.

Principal components analysis using allelic frequencies at eight polymorphic loci revealed similar relationships among collections within stocks and regions (Fig. 5). Sockeye samples from the Egegik and Ugashik rivers were similar, forming a relatively distinct Bristol Bay grouping but this grouping did not include Iliamna Lake. Samples from the Fraser and Columbia rivers formed clusters that overlapped with each other but remained distinct from samples from other regions. Kamchatka River, Babine and Meziadin lakes each formed distinct clusters. Samples from the Iliamna and Kuril stocks were generally very similar, but they were distinct from all other stocks. It should be noted however that the Iliamna and Kuril stocks could be distinguished using allelic frequencies at *PEP-C** (examined in these stocks but not in other Bristol Bay stocks). The frequency of *PEP-C*100* averaged 0.843 in Kuril Lake, but was fixed (1.00) in the few collections examined from Iliamna Lake (Table 1).

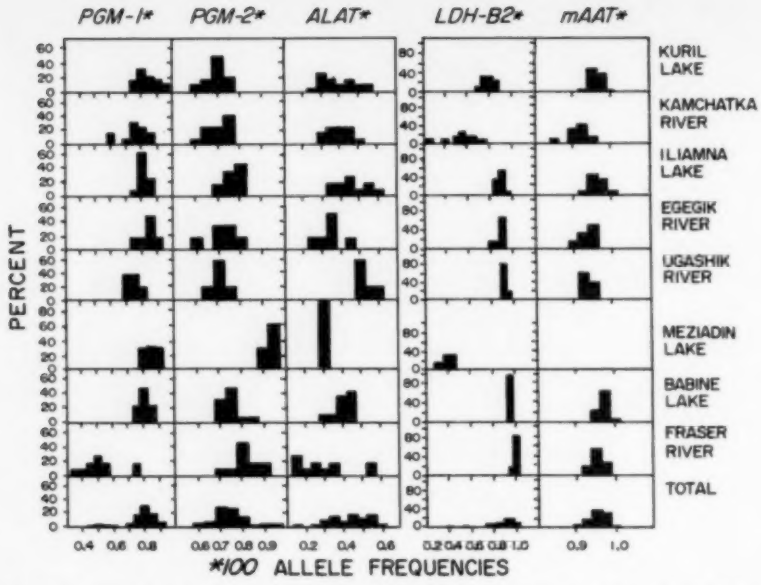

FIG. 2. Distribution of allelic frequencies at the five most polymorphic loci in eight stocks of sockeye salmon from Asia and North America. The vertical axis represents the percentage of samples analyzed.

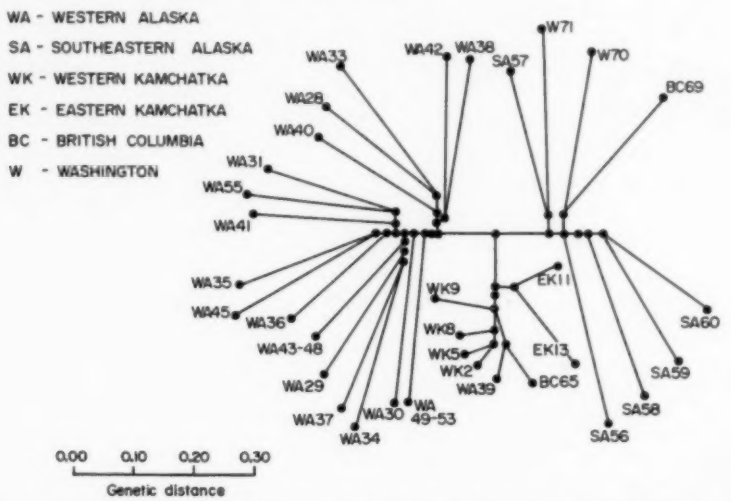

FIG. 3. Neighbor-joining tree based on pooled allelic frequencies at seven polymorphic loci (*LDH-B2**, *PGM-1**, *PGM-2**, *GPI-B1,2**, *MDH-1,2**, *ALAT**, *PEP-LT2**) for 34 sockeye stocks from Asia and North America. Alphabetic characters designate regions; numerals correspond to sites in Table 1.

Discussion

Only a low level of biochemical genetic variation was detected in sockeye salmon. Of 87 loci screened, over 30% were fixed or nearly fixed with extremely low levels of heterozygosity, and only about 15% were polymorphic with frequencies of the common allele <0.99. A similar number of loci have been screened in other Pacific salmon, revealing more variation; about 40% of loci were polymorphic in pink salmon (*O. gorbuscha*), and more than 50% were polymorphic in chum salmon (*O. keta*) (Shaklee and Varnavskaya 1994; Winans et al. 1994). The low level of polymorphism characteristic of sockeye has been discussed previously (Altukhov 1974; Allendorf and Utter 1979; Kirpichnikov 1989), but it has not yet been explained. The inbreeding process seems to be more efficient in sockeye populations than in populations of other Pacific salmon. This is probably related to the greater extent of reproductive isolation between small spawning populations, a consequence of well-developed homing behavior in sockeye salmon as demonstrated by tagging experiments and gene flow calculations (Hartman and Raleigh 1964; Ilin et al. 1983; Varnavsky and Varnavskaya 1985; Quinn et al. 1987).

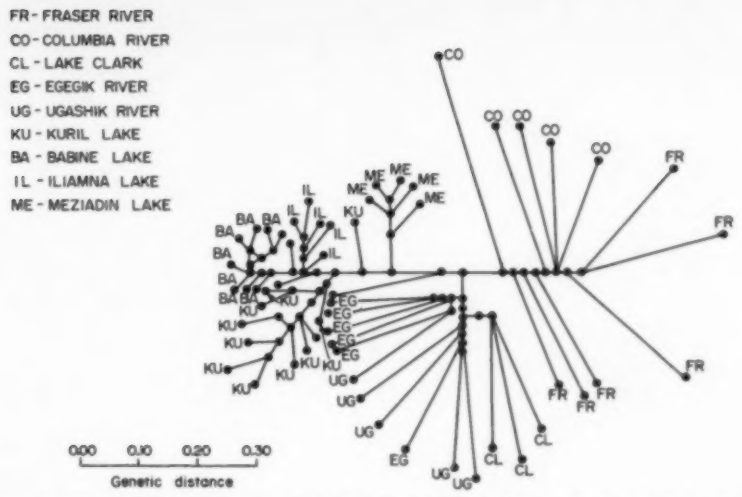

FIG. 4. Neighbor-joining tree based on allelic frequencies at eight polymorphic loci (those in Fig. 3 plus *mAAT-1**) in collections from nine major stocks of sockeye salmon.

On the other hand, a few polymorphic loci exhibit high levels of heterozygosity and variation in allelic frequencies throughout the species' geographic range, compared with other salmonids. In some cases (e.g., *LDH-B2**, *PGM-1**, and *ALAT**), alleles that were dominant in one population were almost absent in another. This large range in allele frequencies was evident not just among geographically remote populations, but sometimes among populations within the same river system. In fact, genetic differentiation was typically greater among populations than among regional groupings of populations. The extent of differentiation among individual populations suggests that existing allozyme allele frequency data could be used to estimate mixed-stock fishery contributions for the major stocks examined in this study. However, more samples should be screened at *PEP-C**, *mAH-1,2**, *mAH-3**, and *mAH-4** to demonstrate the full potential of allozymes for identification of sockeye stocks in the north Pacific Ocean.

Several sockeye populations in Kamchatka and northern British Columbia were distinguished by their high prevalence of *LDH-B2*115*, an allele that was rare or absent in other populations (Table 1). For example, *LDH-B2*115* frequencies ranged from 0.084–0.816 within the Kamchatka River, from 0.048 to 0.587 within the Stikine River and from 0.027 to 0.576 within the Nass River, ranges almost as large as that observed throughout the species' range (Fig. 6, Table 1; the frequency of the other variant, *LDH-B2*85*, never exceeded 0.01 in samples from these rivers). Withler (1985) attributed the high level of variation at *LDH-B2** among North American populations to random genetic drift and colonization events after the late Wisconsin glaciation. However, there is some evidence to suggest that *LDH-B2** is subject to disruptive selection and that *LDH-B2*115* has adaptive value only in environments characterized by low temperature, high dissolved oxygen concentration, and fast current. Kirpichnikov (1977) reported that survival of sockeye fry and embryos exposed to low dissolved oxygen concentrations was negatively associated with the prevalence of *LDH-B2*115*, although too few experiments were performed to demonstrate statistical

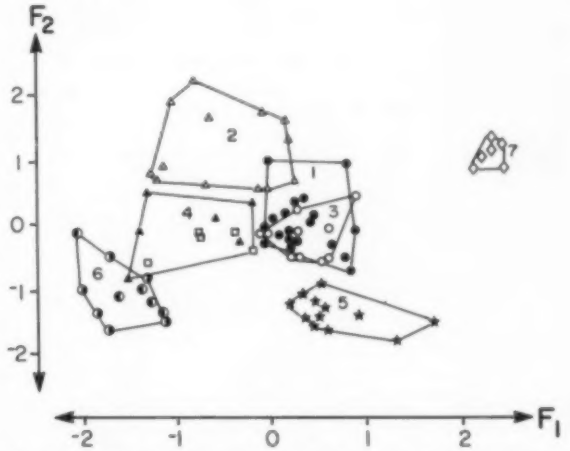

FIG. 5. Principal component analysis of genetic diversity at eight polymorphic loci in eight major sockeye salmon stocks. Numbers label polygons, symbols label stocks: 1 = Kuril Lake (solid circles); 2 = Kamchatka River (open triangles); 3 = Iliamna Lake (open circles); 4 = Bristol Bay region including Egegik River (solid triangles) and Ugashik River (open squares); 5 = Babine Lake (stars); 6 = Fraser (circles open on left) and Columbia (circles open on right) rivers; and 7 = Meziadin Lake (diamonds).

significance. In other experiments, genotypes homozygous for *LDH-B2*100* exhibited higher tolerance to high temperature in vitro than did genotypes heterozygous or homozygous for *LDH-B2*115* (Kirpichnikov 1977, 1989). A significant positive correlation was also observed between the prevalence of *LDH-B2*115* in samples of both juvenile and adult sockeye from subpopulations in Azabache Lake (Kamchatka), and dissolved oxygen concentration on their respective spawning grounds under natural conditions (Novoselskaya et al. 1982). Similarly, a positive correlation was found between water velocity on spawning grounds tributary to Nachiki Lake and *LDH-B2*115* frequencies in samples of adults spawning there (Varnavskaya 1984).

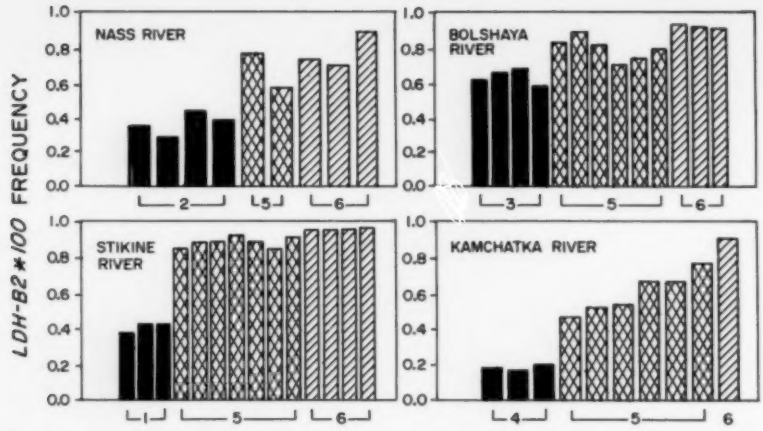

FIG. 6. *LDH-B2*100* frequencies in sockeye salmon populations in large river basins of northern Canada and Russia. This figure contrasts sockeye from upper mountain lakes and their tributaries (1 = Tahltan Lake, Stikine River; 2 = Meziadin Lake, Nass River; 3 = Nachiki Lake, Bolshaya River; 4 = Dvu-Yurta Lake, Kamchatka River) with river-spawning populations (5) and late-run lake-spawning populations (6) in the same drainages.

TABLE 4. Summary of *G*-tests of genetic heterogeneity among samples within regions and major sockeye salmon stocks in Asia and North America. Up to nine polymorphic loci were considered simultaneously. *G*-tests for regions (totals) were calculated using data from all collections analyzed at more than six loci; *G*-tests for major stocks were calculated where at least three collections could be compared within stocks.

Location	df	*G*-test	*P*
West Kamchatka			
Hairusova River	20	60.1	<0.05
Bistraya River	10	10.7	>0.05
Kuril Lake	172	447.5	<0.001
Total	189	611.7	<0.001
East Kamchatka			
Kamchatka River	66	149.5	<0.001
Dalnee Lake	13	29.0	<0.01
Total	88	604.7	<0.001
West Alaska			
Egegik River	30	59.5	<0.01
Ugashik River	22	22.7	>0.05
Iliamna Lake	60	97.7	<0.01
Lake Clark	12	15.4	>0.05
Total	144	2830.4	<0.001
Southeastern Alaska			
Total	20	257.0	<0.001
British Columbia			
Babine Lake	59	98.2	<0.01
Meziadin Lake	26	123.5	<0.001
Total	264	1231.6	<0.001

Several observations from the present study are consistent with the hypothesis that *LDH-B2*100* and *LDH-B2*115* are adaptive in different habitats. *LDH-B2*115* is almost absent in southern (Fraser and Columbia rivers) populations and rare in most Russian populations of late-run sockeye that spawn in oxygen-poor conditions associated with underground springs in littoral areas. On the other hand, the highest frequencies of *LDH-B2*115* all occur in populations associated with cold, high gradient tributaries and mountain lakes, including Nachiki Lake in the Bolshaya River basin and Dvu-Yurta Lake in the Kamchatka River basin (Varnavskaya 1984; Varnavskaya et al. 1988), as well as Tahltan Lake in the Stikine River basin (Wood et al. 1987) and Meziadin Lake in the Nass River basin (present data). Sockeye spawning in Nachiki and Dvu Yurta lakes typically enter fresh water in May and migrate long distances upstream to reach their natal lake at high elevation where they spawn under conditions of low temperatures, high oxygen levels and fast water currents in tributaries. They are considered "early-run sockeye" because they spawn in July. Sockeye spawning in Tahltan and Meziadin lakes enter fresh water in June, and both must ascend very steep streams past waterfalls to reach their natal lakes. The Tahltan migration is earlier than other populations in the Stikine River but the Meziadin migration is similar to most other populations in the Nass River. It is also noteworthy that *LDH-B2*115* frequencies were typically higher in early-run than in late-run sockeye populations in other lakes on both continents where early and late sockeye were examined (e.g., Kronoki Lake, Kamchatka River, Azabache Lake, Russian River) (Table 1). Exceptions to this pattern included Karluk Lake of western Alaska and a few other sites where no significant differences in allelic frequency were detected between early and late sockeye runs.

The pattern of genetic diversity across sockeye populations cannot be explained by random genetic drift or selection of *LDH-B2** alleles alone. Present sockeye population structure is probably the result of interactions among several colonizing groups that survived the last ice age in isolated refugia with different habitat characteristics (Withler 1985; Wood et al. 1994). Sockeye are known to have survived the late Wisconsin glaciation in different types of refugia, primarily those in areas south of the ice sheets (e.g., the Columbia

River), and in arid northern areas that remained largely free of ice (e.g., Beringia, the region connecting Kamchatka and much of western Alaska) (McPhail and Lindsay 1970). They may also have survived in coastal refugia in Canada (Wood et al. 1994) and in small inland mountain refugia where glaciers prevented or impeded access to the sea.

Sockeye populations that survived glaciation in the Columbia refuge were isolated from northern refugia and presumably developed their own adaptations, quite different from those in other parts of the species' range. The Fraser and Columbia rivers were connected as ice retreated following the late Wisconsin glaciation (McPhail and Lindsey 1970) and this accounts for the fact that modern Fraser and Columbia populations are related to each other but distinct from sockeye in other regions (this paper; Wood et al. 1994).

The unusually high prevalence of *LDH-B2*115* in the Tahltan and Meziadin sockeye populations of northern British Columbia is especially puzzling. If *LDH-B2*115* confers a physiological swimming advantage in cold water, it seems plausible that sockeye carrying these alleles may have been more likely to colonize these lakes given the difficulty and extent of upstream migration. Similarly, in the Russian River of the Kenai River basin (Cook Inlet area, Alaska), waterfalls impede upstream migration, especially of early-run sockeye, and LDH-B2*115 frequencies are significantly higher than in other Cook Inlet populations (Wilmot and Burger 1985; Table 1).

We speculate that modern early-run populations of sockeye in Kamchatka, characterized by a high prevalence of *LDH-B2*115* are descended from sockeye that survived in small mountain refugia. The Kamchatka peninsula was only partly covered by ice sheets, and many highland areas in the Central Mountain Ridge remained free of ice but were cut off from the sea by glaciers that formed on both slopes and in river valleys (Braitseva et al. 1968). Nachiki and Dvu-Yurta lakes are situated in areas where such refugia were likely to occur (Braitseva et al. 1968). Early-run sockeye in those lakes are distinct from neighboring populations in allozyme allelic frequencies, age composition and scale patterns (Krogius 1970). In many cases, ecological, biological, and genetic differences between early-run and late-run sockeye within the same river system are greater than those between stocks from different regions (Bugaev 1984; Varnavskaya 1984). Presumably such differentiation would be accelerated by the increased magnitude of random genetic drift in freshwater refugia owing to their small size and strict isolation. Adaptation to unique environmental conditions in the freshwater refugia should also have promoted differentiation from populations in other refugia. Thus, we consider it likely that the genetic structure of modern sockeye populations in Kamchatka and northern Siberia has resulted from interactions between two colonizing races — one originating from populations that survived the last glaciation in inland mountain refugia, and the other, from populations that survived in Beringia or the Okhotsk Sea area with access to the sea. The latter race was probably more abundant and has probably founded most modern populations in Kamchatka and Alaska.

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Genetic Differentiation of Subpopulations of Sockeye Salmon (*Oncorhynchus nerka*) Within Lakes of Alaska, British Columbia, and Kamchatka, Russia

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Genetic differentiation among subpopulations of sockeye salmon (*Oncorhynchus nerka*) was investigated within nine intensively sampled lake systems located throughout the species' range using allozyme allelic frequency data collected by researchers in Canada, Russia, and the United States. Allelic frequencies at up to nine highly polymorphic loci were used to examine genetic diversity among 163 samples collected from 68 distinct spawning sites and to identify subpopulation structure within lakes. Significant heterogeneity was detected among sites within all lakes. The greatest differentiation was evident among subpopulations exhibiting different run timing (earlier vs. later) or utilizing different spawning habitat (tributary vs. littoral). These findings indicate that sockeye home precisely to natal streams, not just to lake systems, and underscore the importance of conserving individual spawning sites within sockeye populations.

Les auteurs ont étudié la différenciation génétique chez des sous-populations de saumon rouge (*Oncorhynchus nerka*) de neuf systèmes lacustres, soumis à un échantillonnage intensif et situés dans l'aire de répartition de l'espèce, en se servant de données sur les fréquences alléliques des allozymes recueillies par des chercheurs du Canada, de la Russie et des États-Unis. Ils ont utilisé les fréquences alléliques en jusqu'à neuf loci fortement polymorphes pour examiner la diversité génétique chez 163 échantillons prélevés dans 68 différentes frayères et préciser la structure des sous-populations de chaque lac. Ils ont décelé une hétérogénité significative entre les sites de chaque lac. La plus grande différenciation a été notée chez les sous-populations qui reviennent frayer à des moments différents (montaison hâtive v. montaison tardive) ou qui utilisent des habitats différents pour frayer (tributaire v. littoral). Cela montre que le saumon rouge retourne sans difficulté à son cours d'eau natal, et non seulement au système lacustre d'origine, et souligne l'importance de protéger individuellement les frayères fréquentées par les diverses composantes de chaque population.

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The size and distinctiveness of population units have always been key problems in understanding the evolution of organisms. Knowledge about population structure is also required for successful exploitation and conservation of valuable species such as Pacific salmon (Oncorhynchus spp.). Pacific salmon, especially sockeye salmon (O. *nerka*), are known for their precise homing behavior; mature fish return to spawn near the place of their early freshwater life (Hasler and Scholz 1983; Quinn 1985, 1993). For this reason, it is generally accepted that sockeye salmon returning to different lakes belong to distinct population units (e.g., Wood et al. 1994). Quinn et al. (1987) used differences in allozyme allelic frequencies and parasite

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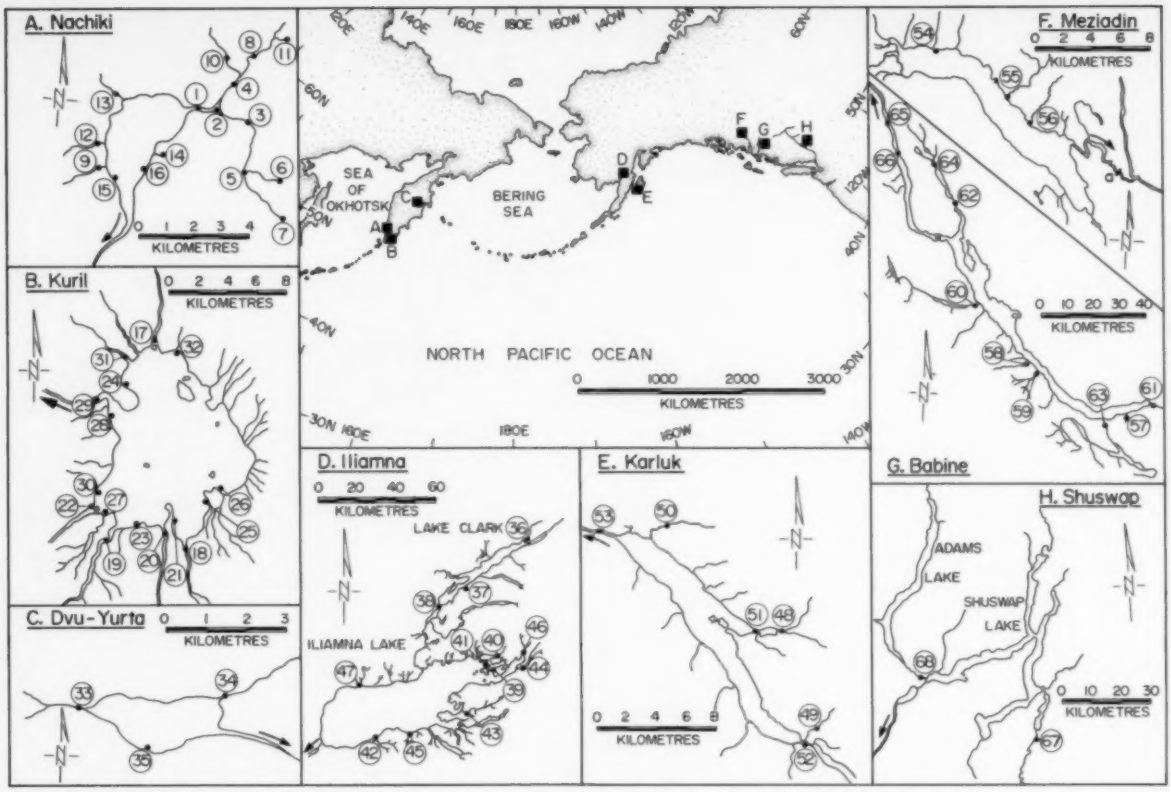

FIG. 1. Geographic distribution of the nine study lakes (A–H), and location of sampling sites within each lake. Site numbers 1–68 correspond to those listed in Tables 1 and 4. Site a in frame F is the fishway to Meziadin Lake.

prevalence to demonstrate that fewer than 1% of sockeye strayed between neighbouring lakes in the same river system on Vancouver Island and between nearby river systems on the mainland coast of British Columbia.

Precise homing could also lead to reproductive isolation and eventually to genetic differentiation among spawning sites within lake systems, but such precise homing has not been well documented. Direct measurement of homing to natal spawning sites has been impractical, and to our knowledge the only direct evidence for the precision of homing comes from studies in which the movements of tagged adults were monitored after they had been displaced from spawning streams in Karluk (Hartman and Raleigh 1966), Babine (McCart 1970), Azabache (Ilin et al. 1983), Nachiki (Varnavsky and Varnavskaya 1985), and Iliamna (Blair and Quinn 1991) lakes. These studies indicated that straying among spawning sites within lakes averaged 2–5%, except between islands in Iliamna Lake where less homing was observed. However, the reliability of estimates obtained from displacement experiments has been disputed (Ricker 1972), and some researchers have suggested that sockeye populations within some nursery lakes are subdivided into genetically distinct "subpopulation" units, typically comprising only 300–500 spawners with a mean effective population size of perhaps <200 (Altukhov 1974; Konovalov 1980).

To investigate the extent of genetic differentiation among sockeye subpopulations in Lake Azabache, Altukhov (1974) and Altukhov et al. (1975) analyzed variation at two biochemical loci coding the enzymes lactate dehydrogenase (*LDH-B2**) and phosphoglucomutase (*PGM-2**). Although only two loci were examined, significant genetic variation was detected among subpopulations, including those spawning in large rivers, small creeks, and littoral areas of Lake Azabache. Such differentiation among subpopulations was not always detected in similar studies of sockeye in other Kamchatka lakes (Varnavskaya et al. 1988); in some lakes sockeye spawning in different littoral sites appeared genetically similar and the authors concluded that straying among spawning sites probably exceeded 2–5%. Of course, genetic diversity could not be estimated reliably with data from only the two loci studied at that time.

In this paper we investigate the genetic differentiation of sockeye subpopulations within nine lakes located throughout the species' range (Fig. 1) using data collected between 1978 and 1991 by researchers in Canada, Russia, and the United States. Assuming that these well-studied lakes are representative of other major sockeye-producing lakes, we draw conclusions about patterns of subpopulation structure in sockeye lakes. These findings have important implications for the precision of homing in sockeye and for fisheries management.

Materials and Methods

We compiled genetic data collected between 1978 and 1991 from 163 samples of sockeye salmon from nine lake systems

TABLE 1. Location and characteristics of sockeye spawning sites sampled. Site numbers correspond to those in Fig. 1.

		Ecological type			
Region	Nursery lake	Spawning habitat[a]	Period of peak spawning	Relative timing of spawning	Site nos.
Kamchatka	Nachiki	Tributary	July	Earlier	1–14
		Lake	Aug.–Sept.	Later	15,16
	Kuril	Tributary	Jul.–Sept.	Earlier	17–20,22,31
			Oct.–Dec.	Later	19,20,22
		Outlet	Sept.–Oct.	Later	29
		Lake	Aug.–Sept.	Earlier	21,23–28,32
			Oct.–Dec.	Later	23,24,26–28,30,32
	Dvu-Yurta	Tributary	July	—	33,34
		Lake	July	—	35
Alaska	Clark	Lake	Aug.–Sept.	—	36–38
	Iliamna	Lake	Aug.–Sept.	—	39–41
		Tributary	Aug.–Sept.	—	42–47
	Karluk	Tributary	July–Aug.	Earlier	48–50
			Aug.–Sept.	Intermediate	51–53
		Outlet	Sept.–Oct.	Later	
Canada	Meziadin	Lake	Aug.–Sept.	—	54
		Tributary	Aug.–Sept.	—	55–56
	Babine	Tributary	Aug.	Earlier	57–59
			Sept.	Intermediate	60–64
		Outlet	Sept.–Oct.	Later	65–66
	Shuswap	Tributary	Oct.	—	67–68

[a]tributary = inlet tributary to lake; outlet = outlet tributary; lake = littoral spawning sites.

TABLE 2. Enzymes and polymorphic loci used for analysis.

Enzyme	IUBNC number	Locus	Tissue[a]	Allele[b] mobilities				
Lactate dehydrogenase	1.1.1.27	*LDH-B2**	M, H, E	100	115	85		
Malate dehydrogenase	1.1.1.37	*MDH-B1,2**	M, H, E	100	65	120	60	
Alanine aminotransferase	2.6.1.2	*ALAT**	M	100	91	95	86	108
Glycyl-leucine peptidase	3.4	*PEP-C**	E	100	105			
Leucyl-tyrosine peptidase	3.4	*PEP-LT2**	M, H	100	92	114		
Phosphoglucomutase	5.4.2.2	*PGM-1**	M, H, E	−100	0			
		*PGM-2**	M, H, E	−100	−77	−93	−125	
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B1,2**	M	100	132	143		
Aspartate aminotransferase	2.6.1.1	*mAAT-1**	M, H	−100	-82			

[a]Tissue designations are: M = muscle; H = heart; E = eye retina.
[b]Buffer systems and level of variation are described by Varnavskaya et al. (1994) and Wood et al. (1994).

(Table 1). Samples from Karluk Lake (Kodiak Island, Alaska), Iliamna Lake and Lake Clark (Bristol Bay, Alaska) were collected and examined by staff of the U.S. Fish and Wildlife Service Research Office, Anchorage, AK; the Pacific Biological Station, Nanaimo, B.C.; and the Fisheries Research Institute, Seattle, WA. Collections from Meziadin, Babine, and Shuswap lakes (British Columbia) were collected and analyzed by staff of the Pacific Biological Station, Nanaimo, B.C.; samples from Kuril, Nachiki, and Dvu-Yurta lakes (Russia) were collected and analyzed by staff of the Kamchatka Research Institute of Fisheries and Oceanography (KoTINRO) in conjunction with staff at the Pacific Biological Station, Nanaimo, B.C., and U.S. Fish and Wildlife Research Office, Anchorage, AK. Preliminary studies, sample collection and analytical procedures, and allelic frequency data for all major sockeye salmon populations are documented by Varnavskaya et al. (1994) and Wood et al. (1994). To ensure consistency of data collected by different agencies, many of the samples from Canada, Russia, and the United States were analyzed cooperatively, and scored simultaneously on the same gels. Although the number of loci screened for genetic variation has increased from only 2–3 loci before 1982 to as many as 87 for some samples by 1990, most of the additional loci are monomorphic or reveal little genetic variation (Varnavskaya et al. 1994). In the present analysis of subpopulation structure, we have focused on genetic variation at up to nine polymorphic loci (Table 2).

We used likelihood ratio analysis (*G*-test; Sokal and Rohlf 1981) to test for heterogeneity in allelic frequencies among different samples collected from the same lake in the same year. Where possible, we also examined heterogeneity within and among "ecological types" exhibiting different spawning

TABLE 3. Average allelic frequencies, levels of population subdivision (F_{ST}), and results of G-tests for heterogeneity of allelic frequencies among sites within lakes. N = number of sites within lake. Significance of G-test is shown by asterisks following the F_{ST} values[a].

(A) *LDH-B2** and *PGM-2**.

			*LDH-B2**		*PGM-2**	
Lake	Year	N	**100*	F_{ST}[a]	**100*	F_{ST}
Nachiki	1979	2	0.903	0.002	0.737	0.002
	1980	13	0.672	0.021	0.669	0.006
	1981	14	0.667	0.030***	0.660	0.007
	1982	9	0.741	0.083***	0.685	0.007
	1983	2	0.923	0.009	0.705	0.000
	Total	40	0.710	0.063***	0.675	0.008
Kuril	1989	23	0.816	0.027***	0.724	0.012*
	1990	18	0.814	0.014	0.717	0.011
	Total	41	0.815	0.021***	0.721	0.012
Dvu-Yurta	1982	3	0.177	0.008	0.803	0.006
Clark	1988	3	0.850	0.002	0.783	0.010
Iliamna	1988	5	0.902	0.009	0.773	0.004
	1991	6	0.910	0.007	0.804	0.008
	Total	11	0.906	0.008	0.790	0.008
Karluk	1978	2	0.877	0.018	0.779	0.020*
	1979	2	0.870	0.008	0.751	0.001
	1980	2	0.926	0.003	0.844	0.007
	1981	5	0.868	0.016**	0.746	0.003**
	1982	6	0.876	0.012**	0.743	0.003
	1983	6	0.893	0.010*	0.771	0.018*
	1984	6	0.884	0.009	0.831	0.016**
	1985	6	0.904	0.017***	0.789	0.012*
	Total	35	0.886	0.015***	0.779	0.003***
Meziadin	1982	1	0.434	—	0.955	—
	1984	1	0.430	—	0.935	—
	1986	3	0.401	0.020**	0.960	0.005
	1987	1	0.475	—	0.970	—
	Total	6	0.424	0.013**	0.958	0.006
Babine	1985	4	0.969	0.004	0.795	0.004
	1987	9	0.973	0.007	0.760	0.003
	1988	5	0.972	0.003	0.778	0.002
	1990	2	0.972	0.000	0.770	0.002
	Total	20	0.972	0.005	0.774	0.003
Shuswap	1983	1	0.995	—	0.842	—
	1986	1	0.990	—	0.870	—
	1990	2	0.998	0.003	0.877	0.015*
	Total	4	0.994	0.003	0.867	0.009*

[a]Significance levels: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

times or utilizing different spawning habitat (river vs. littoral) within the lake in the same year. Differences in seasonal timing at spawning sites within lakes were defined by the relative terms "earlier", "intermediate", or "later" to avoid confusion with the terms "early run" and "late run," which are sometimes used to designate absolute run timing (e.g., Varnavskaya et al. 1994). Wright's (1951) F_{ST} and other statistics describing allelic frequency variation among samples were calculated using the BIOSYS-1 computer program (Swofford and Selander 1981). Rare alleles were pooled for statistical analyses of variation at all loci except at *ALAT**, where *ALAT*86* and *ALAT*108* were pooled for F_{ST} calculations and *ALAT*86*, *ALAT*108*, and *ALAT*95* were pooled for G-tests. The effective number of migrants exchanged among sites per generation (Nm) was calculated from F_{ST} values averaged across all polymorphic loci using the approximate relationship $F_{ST} = 1/(4Nm + 1)$ as described by Allendorf and Phelps (1981). Schedules for this symposium did not permit more detailed tests of panmixia using data on genotypic and gametic phase disequilibria.

Results

Allelic frequencies at *LDH-B2**, *PGM-1**, *PGM-2**, *GPI-B1,2**, *mAAT-1**, *ALAT**, *MDH-B1,2**, *PEP-LT2**, and *PEP-C** showed considerable variation both within and among lakes. Annual variability in allelic frequencies within lakes was low and differences among lakes were generally

TABLE 3. *(Continued)*

(B) *PGM-1**, *ALAT**, *GPI-B1,2**, and *MDH-B1,2**.

		*PGM-1**			*ALAT**			*GPI-B1, 2**			*MDH-B1, 2**		
Lake	Year	*N*	**null*	F_{ST}[a]	*N*	**100*	F_{ST}	*N*	**null*	F_{ST}	*N*	**100*	F_{ST}
Kuril	1989	23	0.835	0.029***	23	0.432	0.004***	23	0.998	0.008	23	0.999	0.008
	1990	18	0.853	0.028***	18	0.412	0.008***	18	0.998	0.024	18	0.998	0.018
	Total	41	0.842	0.029***	41	0.424	0.006***	41	0.998	0.016	41	0.999	0.016
Clark	1988	3	0.687	0.008	3	0.688	0.025	3	1.000	—	3	1.000	—
Iliamna	1988	5	0.849	0.005	5	0.436	0.013***	5	1.000	—	5	0.998	0.008
	1991	6	0.821	0.003	6	0.388	0.016	6	1.000	—	6	1.000	—
	Total	11	0.834	0.005	11	0.410	0.017***	11	1.000	—	11	0.999	0.009
Meziadin	1982	1	0.826	—	—	—	—	1	1.000	—	1	1.000	—
	1984	1	0.890	—	—	—	—	1	1.000	—	1	1.000	—
	1986	3	0.904	0.001	3	0.377	0.002	3	1.000	—	3	1.000	—
	1987	1	0.840	—	1	0.325	—	1	1.000	—	1	1.000	—
	Total	6	0.876	0.010	4	0.335	0.004	6	1.000	—	6	1.000	—
Babine	1985	4	0.787	0.001	1	0.388	—	—	—	—	—	—	—
	1987	9	0.813	0.007	9	0.478	0.011***	8	1.000	—	8	1.000	—
	1988	5	0.817	0.007	5	0.457	0.003	4	1.000	—	5	1.000	—
	1990	2	0.817	0.000	2	0.372	0.003*	2	1.000	—	2	1.000	—
	Total	20	0.809	0.006	17	0.435	0.010***	14	1.000	—	14	1.000	—
Shuswap	1983	1	0.586	—	1	0.786	—	—	—	—	—	—	—
	1986	1	0.417	—	1	0.688	—	1	0.997	—	1	0.975	—
	1990	2	0.329	0.019**	2	0.681	0.015***	2	0.998	0.003	2	0.965	0.036***
	Total	4	0.415	0.054***	4	0.709	0.015***	3	0.997	0.002	3	0.968	0.026***

[a]Significance levels: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

TABLE 3. *(Concluded)*

(C) *PEP-C*, *mAAT-1**, and *PEP-LT**.

		*PEP-C**			*mAAT-1**			*PEP-LT**		
Lake	Year	*N*	**100*	F_{ST}[a]	*N*	**-100*	F_{ST}	*N*	**100*	F_{ST}
Kuril	1989	—	—	—	5	0.944	0.024***	20	0.998	0.016
	1990	13	0.857	0.025***	8	0.945	0.012	18	0.998	0.017
	Total	13	0.857	0.025***	3	0.945	0.018***	38	0.998	0.017
Clark	1988	—	—	—	3	0.897	0.025*	3	1.000	—
Iliamna	1988	—	—	—	5	0.924	0.004	5	1.000	—
	1991	6	0.989	0.038**	6	0.954	0.024	6	1.000	—
	Total	6	0.989	0.038**	1	0.941	0.016**	11	1.000	—
Meziadin	1986	3	0.995	0.003	—	—	—	—	—	—
Babine	1985	1	0.989	—	—	—	—	—	—	—
	1988	5	0.922	0.012*	—	—	—	—	—	—
	1990	—	—	—	2	0.961	0.003	2	0.965	0.001
	Total	6	0.933	0.022***	2	0.961	0.003	2	0.965	0.001
Shuswap	1983	1	1.000	—	—	—	—	—	—	—
	1986	1	1.000	—	—	—	—	—	—	—
	1990	2	1.000	—	—	—	—	—	—	—
	Total	4	1.000	—	—	—	—	—	—	—

[a]Significance levels: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

consistent from year to year (Table 3). Statistically significant heterogeneity in allelic frequencies was observed within years among some collections in most lakes, especially at *LDH-B2**, *ALAT**, and *PEP-C** (Table 3, Appendix).

Variation at *LDH-B2** was highly significant within years in Kuril ($P < 0.001$, $F_{ST} = 0.027$), Nachiki ($P < 0.001$, $F_{ST} > 0.030$), Karluk ($P < 0.001$, $F_{ST} = 0.017$), and Meziadin ($P < 0.01$, $F_{ST} = 0.020$) lakes, but not within Lake Clark, Dvu-Yurta, Iliamna, Babine, or Shuswap lakes ($F_{ST} < 0.009$) (Table 3A). Variation among sites was greatest in lakes where earlier and later runs occurred (as in Kuril, Nachiki, and Karluk lakes).

TABLE 4. Comparison of heterogeneity in allelic frequencies (all polymorphic loci) within and among groupings based on ecological type. The effective number of migrants per generation (Nm) was calculated from the F_{ST} values; significance levels for G-tests as in Table 3.

Lake	Year	Source of variation	No. of samples	F_{ST}	G	df	Nm
Nachiki	1982	Within earlier-tributary type	7	0.018	14.5	18	13.6
		Within later-lake type	2	0.011	1.4	3	22.5
		Between types	2	0.042	32.9***	3	5.7
		Total	9	0.043	48.8**	24	5.6
Kuril	1989	Within earlier-tributary type	6	0.009	858.9***	40	27.5
		Within earlier-lake type	7	0.020	142.3***	42	12.3
		Within later-tributary type	4	0.018	59.2***	24	13.6
		Within later-lake type	5	0.026	111.9***	32	9.4
		Among types	5	0.007	154.9***	28	35.5
		Total	23	0.023	1192.9***	154	10.6
	1990	Within earlier-tributary type	8	0.022	224.8***	63	11.1
		Within earlier-lake type	2	0.016	10.2	9	15.4
		Within later-tributary type	2	0.007	24.4**	10	35.5
		Within later-lake type	5	0.018	67.3**	36	13.6
		Among types	5	0.022	566.3***	36	11.1
		Total	18	0.023	413.8***	153	10.6
Iliamna	1991	Within lake type	3	0.004	13.5	12	62.2
		Within tributary type	3	0.006	16.1	12	41.1
		Between types	2	0.007	20.6**	6	35.5
		Total	6	0.011	50.2*	30	22.5
Karluk	1978	Between sites–types	2	0.019	7.9*	2	12.9
	1979	Between sites–types	2	0.004	3.8	2	62.2
	1980	Between sites–types	2	0.006	31.5***	2	41.4
	1981	Within earlier type	3	0.002	2.1	4	124.8
		Within intermediate type	2	0.002	27.5***	2	124.8
		Between types	2	0.006	35.5***	2	41.4
		Total	5	0.008	65.2***	8	31.0
	1982	Within earlier type	3	0.007	9.8*	4	35.5
		Within intermediate type	2	0.000	1.1	2	—
		Among types	3	0.014	10.0*	4	17.6
		Total	6	0.007	22.8*	10	35.5
	1983	Within earlier type	3	0.013	22.7***	4	19.0
		Within intermediate type	2	0.000	2.1	2	—
		Among types	3	0.011	13.8**	4	22.5
		Total	6	0.015	38.6***	10	16.4
	1984	Within earlier type	3	0.004	6.8	4	62.2
		Within intermediate type	2	0.006	4.2	2	41.4
		Among types	3	0.012	17.5**	4	20.6
		Total	6	0.013	28.5**	10	19.0
	1985	Within earlier type	3	0.007	7.5	4	35.5
		Within later type	2	0.002	1.2	2	124.8
		Among types	3	0.014	29.1***	4	17.6
		Total	6	0.014	37.8***	10	17.6
Meziadin	1986	Within tributary type	2	0.003	4.4	3	83.1
		Between lake–tributary types	2	0.007	17.8***	3	35.5
		Total	3	0.009	22.2**	6	27.5
Babine	1985	Within earlier type	2	0.003	3.4	3	83.1
		Within intermediate type	2	0.001	1.3	3	249.8
		Between types	2	0.000	1.5	3	—
		Total	4	0.002	6.3	9	124.8
	1987	Within earlier type	3	0.002	9.0	10	124.8
		Within intermediate type	4	0.010	40.7***	15	24.8
		Within later type	2	0.004	12.3*	5	62.2
		Among types	3	0.002	12.0	10	124.8
		Total	9	0.007	74.0***	40	35.5

TABLE 4. *(Concluded)*

Lake	Year	Source of variation	No. of samples	F_{ST}	G	df	Nm
	1988	Within earlier type	3	0.002	16.5	12	124.8
		Within intermediate type	2	0.002	5.0	6	124.8
		Between types	2	0.002	6.0	6	124.8
		Total	5	0.004	27.6	24	62.2
	1990	Within intermediate type	2	0.002	35.4***	7	124.8

*PGM-1** and *PGM-2** exhibited lower levels of variation in allelic frequency among sites within years (Tables 3A and 3B). Significant variation was detected among sites at *PGM-1** only in Kuril ($P < 0.001$, $F_{ST} > 0.028$) and Shuswap ($P < 0.01$, $F_{ST} = 0.019$) lakes, and at *PGM-2** in Karluk ($P < 0.01$, $F_{ST} = 0.016$), Kuril ($P < 0.05$, $F_{ST} = 0.012$), and Shuswap ($P < 0.05$, $F_{ST} = 0.015$) lakes.

Heterogeneity among subpopulations at *ALAT** was highly significant within years in Kuril, Iliamna, Babine, and Shuswap lakes ($P < 0.001$, $F_{ST} > 0.008$), marginally significant ($P = 0.055$, $F_{ST} = 0.025$) in Lake Clark, but not significant in Meziadin Lake ($P > 0.25$, $F_{ST} = 0.002$) (Table 3B).

*PEP-C**, *mAAT-1**, and *PEP-LT2** were examined in fewer samples because techniques for assaying variation at these loci were developed more recently (Table 3C). However, significant variation in allelic frequencies at *PEP-C** was detected within years in Kuril ($P < 0.001$, $F_{ST} = 0.025$), Iliamna ($P < 0.01$, $F_{ST} = 0.038$), and Babine ($P < 0.05$, $F_{ST} = 0.012$) lakes. Significant variation among sites within years was detected at *mAAT-1** in Kuril Lake ($P < 0.001$, $F_{ST} = 0.024$), Iliamna Lake ($P < 0.01$, $F_{ST} = 0.024$), and Lake Clark ($P < 0.05$, $F_{ST} = 0.025$), but not within Babine Lake ($P > 0.10$, $F_{ST} = 0.003$).

When all loci were considered together, significant differences were detected among sites within years in all lakes (Table 4). Much of the heterogeneity in allelic frequencies observed within lakes was associated with differences in run timing or spawning habitat (i.e., ecological type). In many cases, greater differences in allelic frequency were observed among different ecological types than among sites within the same ecological type. One important exception to this pattern was Babine Lake where highly significant heterogeneity was observed only within the intermediate timing group that includes samples from Fulton River (site No. 60) and Pinkut Creek (site No. 63), both of which have been enhanced by spawning channel construction and flow control since the 1960s (West and Mason 1987).

The estimated number of migrants (Nm) exchanged among sites on average each generation ranged from 5.6 within Nachiki Lake to 124.8 within Babine Lake (Table 4). In Nachiki, Iliamna, Karluk, and Meziadin lakes, Nm was lower among ecological types (range 5.7–41.4) than within ecological types (range 13.6–124.8). The exceptions to this pattern were Kuril and Babine lakes. In Kuril Lake, Nm was generally low (9.4–35.5) both within and among ecological types, whereas in Babine Lake $Nm > 62$ except within the intermediate timing group in 1987 where $Nm = 24.8$.

Discussion

Evidence for strict homing behavior and reproductive isolation of sockeye populations inhabiting different lakes has been reported by many investigators (e.g., Hamamura 1966; Foerster 1968; Quinn et al. 1987). However, population structuring and the precision of homing to natal streams within nursery lakes has received less attention. By examining patterns of allelic variation at numerous highly polymorphic loci sampled repeatedly in sites within several large lakes, we conclude that sockeye often exist as genetically discrete subpopulations within the same lake. Our results corroborate earlier studies of particular lakes (e.g., Altukhov 1974; Altukhov et al. 1975, 1983; Wilmot and Burger 1985; Varnavskaya et al. 1988; Wood et al. 1994).

For significant heterogeneity in allelic frequencies to arise and be maintained among relatively large spawning runs ($N \gg 100$), gene flow among sites must be restricted (e.g., Allendorf and Phelps 1981). Although our estimates of Nm are based on relatively few polymorphic loci (in some cases only two), and should be interpreted cautiously, they indicate that the proportion of exchange (m) among ecological types is often very low. For example, the proportion of exchange among lake spawning and tributary spawning groups in Nachiki, Meziadin, and Iliamna lakes is on the order of 0.004–0.006 assuming effective group sizes of at least 1000, 10 000, and 100 000, respectively. Estimated rates of exchange among ecological types in Karluk Lake ranged from 0.004–0.012 overall, assuming group sizes of at least 5000, but were lower (<0.005) from 1982–86 when data for the later, outlet tributary site were included in the analysis. The magnitude and consistency of differences among ecological types in these lakes indicates that at least some of these groupings should be considered as partially reproductively isolated subpopulations. The differentiation of subpopulations within Karluk Lake is further evidenced by genetic differences in the migratory behaviour of fry produced by inlet and outlet tributary spawners (Raleigh 1967), and in other lakes by consistent variation in a suite of life-history traits (age, size, fecundity, and egg size) and morphology seen in Iliamna Lake (Blair et al. 1993) and the Wood River Lakes (Wetzel 1993).

Although highly significant heterogeneity was observed among sites in Kuril Lake, the pattern of differences remains unclear. Differences in allelic frequencies among sites were often inconsistent in direction and magnitude from 1989 to 1990. Moreover, the level of differentiation within ecological types was sometimes as high or higher than among types. This suggests that we have failed to define subpopulations correctly in Kuril Lake; estimated exchange rate among sites within ecological types was less than 0.036 in all cases, assuming effective group sizes of at least 1000.

Genetic differences within the intermediate run timing type in Babine Lake in 1987 and 1990 indicate that gene flow is restricted between the large runs to Pinkut Creek and Fulton River ($m < 0.001$ with $N > 100.000$), and perhaps

that allelic frequencies are influenced in some years by enhancement activities at these sites. Rather surprisingly, we failed to detect significant differentiation among ecological types in Babine Lake, especially in 1987 when data from the later, outlet tributary type were available for analysis.

The fine structure of populations indicated by genetic and life-history data, and by experimental displacement of adults (reviewed earlier), demonstrates that sockeye salmon can identify their natal spawning site, and indicates that they imprint as alevins or immediately after emergence. Although such a conclusion is consistent with evidence that sockeye salmon can learn odors at these early stages (e.g., Brannon 1972; Bodznick 1978), it runs counter to the generalization that salmon imprinting takes place at or near the smolt transformation stage (Hasler and Scholz 1983). If imprinting occurs only at the smolt stage in sockeye, then differentiation within lakes would not be observed because sockeye generally vacate their incubation sites to rear within the lake basin for 1 or 2 yr before smolting.

Other species of salmon may also leave the incubation area and rear elsewhere in freshwater prior to smolt transformation (e.g., coho salmon (*O. kisutch*), Peterson 1982; chinook salmon (*O. tshawytscha*) Murray and Rosenau 1989) but the patterns of fry movement and adult homing are not as well documented as in sockeye. Pink (*O. gorbuscha*) and chum (*O. keta*) salmon also migrate downstream at or shortly after emergence and must imprint as alevins or newly emerged fry. Thus, while salmon transplanted from one river to another at or prior to the smolt stage generally return to the site where they were released as opposed to their rearing site (Quinn 1993), under natural conditions salmon imprinting should be assumed to begin at the incubation or emergence stages.

In addition to the implications of our findings for the genetic structure of sockeye salmon populations and for the process of olfactory learning, our results underscore the need to consider the spawning site the fundamental unit for conservation, in the absence of evidence to the contrary. Geographical constraints and the timing of adult migrations sometimes make it difficult to manage fisheries on individual breeding populations. For example, sockeye returning to the roughly 100 spawning sites in the Iliamna Lake – Lake Clark watershed are subdivided into at least three major subpopulations, but because these runs show little temporal separation they are managed as one unit (Jensen and Mathisen 1987). However, mixed-stock fisheries run the risk of overexploiting less productive components, and management strategies should include the identification and conservation of subpopulation structure.

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Appendix

Allelic frequencies in sockeye salmon collections by site and year. Site numbers correspond to those in Figure 1 and Table 1.

A. Collections assayed at only 2 polymorphic loci (1978–1985).

Year	Site Number	LDH-B2* *100 (N)	PGM-2* *100 (N)	Year	Site Number	LDH-B2* *100 (N)	PGM-2* *100 (N)
Karluk Lake				**Nachiki Lake**			
1978	48	0.908 (19)	0.839(105)	1979	15	0.891 (55)	0.718 (55)
	51	0.833 (60)	0.720 (59)		16	0.918 (41)	0.753 (41)
1979	48	0.900(110)	0.736(110)	1980	1	0.724 (49)	0.663 (49)
	51	0.840(100)	0.765(100)		2	0.733 (45)	0.645 (45)
1980	48	0.913 (86)	0.875 (32)		3	0.674 (43)	0.640 (43)
	51	0.940 (75)	0.813 (75)		4	0.772 (46)	0.728 (46)
1981	48	0.893(103)	0.728(103)		5	0.611 (45)	0.600 (45)
	49	0.900(105)	0.767(105)		6	0.585 (41)	0.622 (41)
	50	0.911 (96)	0.719 (96)		7	0.625 (12)	0.667 (12)
	51	0.804(102)	0.777(101)		8	0.804 (28)	0.679 (28)
	52	0.830(100)	0.735(100)		9	0.575 (20)	0.675 (20)
1982	48	0.870(100)	0.700(100)		10	0.639 (54)	0.704 (54)
	49	0.899 (99)	0.742 (99)		11	0.661 (31)	0.675 (31)
	50	0.944 (99)	0.775(100)		12	0.696 (28)	0.733 (28)
	51	0.865(100)	0.762(101)		13	0.644 (18)	0.667 (18)
	52	0.845(100)	0.755(100)	1981	1	0.632 (38)	0.644 (38)
	53	0.835(100)	0.722 (99)		2	0.721 (52)	0.657 (52)
1983	48	0.838 (99)	0.712 (66)		3	0.661 (56)	0.696 (56)
	49	0.930(100)	0.775 (91)		4	0.611 (36)	0.625 (36)
	50	0.925(100)	0.805(100)		5	0.759 (64)	0.680 (64)
	51	0.905(100)	0.765 (83)		6	0.694 (54)	0.676 (54)
	52	0.885(100)	0.755(100)		7	0.548 (42)	0.631 (42)
	53	0.875(100)	0.873 (75)		8	0.813 (39)	0.615 (39)
1984	48	0.860(100)	0.815(100)		9	0.653 (39)	0.602 (39)
	49	0.896(101)	0.797(101)		10	0.686 (51)	0.764 (51)
	50	0.935(100)	0.785(100)		11	0.647 (24)	0.676 (24)
	51	0.839 (66)	0.803 (66)		12	0.608 (37)	0.649 (37)
	52	0.875(100)	0.873 (75)		13	0.797 (32)	0.687 (32)
	53	0.895(100)	0.915(100)		14	0.516 (31)	0.645 (31)
1985	48	0.894 (99)	0.805(100)	1982	1	0.614 (21)	0.714 (21)
	49	0.850(100)	0.720(100)		2	0.671 (38)	0.684 (38)
	50	0.904 (99)	0.786 (98)		4	0.640 (25)	0.640 (25)
	51	0.955 (99)	0.755 (98)		5	0.651 (23)	0.627 (23)
	52	0.950(100)	0.800(100)		7	0.596 (20)	0.642 (20)
	53	0.869 (99)	0.867 (83)		11	0.775 (29)	0.724 (29)
					13	0.839 (31)	0.661 (31)
Dvu-Yurta Lake					15	0.922 (32)	0.719 (32)
1982	33	0.224 (38)	0.763 (38)		16	0.944 (37)	0.741 (37)
	34	0.146 (58)	0.836 (58)	1983	15	0.897 (49)	0.704 (49)
	35	0.164 (37)	0.811 (37)		16	0.948 (29)	0.707 (29)

B. Collections assayed at up to nine polymorphic loci (1982–1990).

Year	Time of sampling	Site number	LDH-B2* *100	PGN-1* *null	PGM-2* *100	GPI-B* *100	mAAT-1* *100	ALAT* *100	ALAT* *91	ALAT* *95	HBB-S1,2* *100	PEP-LT3* *100	PEP-C* *100	
	Kuril Lake													
1989	Aug.	17	0.870 (22)	0.886 (23)	0.761 (21)	1.000 (23)	0.891 (23)	0.478	0.500 (21)	0.000	1.000 (23)	1.000 (23)	-	a
	Aug.	17	0.864 (162)	0.898 (161)	0.775 (162)	1.000 (160)	-	0.378	0.511 (161)	0.067	1.000 (160)	1.000 (122)	-	
	Aug.	18	0.822 (146)	0.845 (58)	0.682 (151)	1.000 (158)	0.992 (62)	0.476	0.423 (143)	0.041	1.000 (158)	1.000 (156)	-	
	Aug.	19	0.820 (97)	0.853 (17)	0.705 (97)	1.000 (97)	0.925 (40)	0.412	0.488 (97)	0.063	1.000 (98)	1.000 (96)	-	
	Nov.	19	0.910 (50)	0.812 (24)	0.730 (50)	1.000 (50)	0.940 (50)	0.359	0.577 (39)	0.013	1.000 (50)	1.000 (50)	-	
	Aug.	20	0.874 (235)	0.875 (248)	0.724 (248)	1.000 (80)	0.931 (160)	0.373	0.569 (239)	0.032	0.997 (80)	1.000 (80)	-	
	Oct.	20	0.750 (50)	0.847 (49)	0.765 (49)	0.990 (50)	0.960 (50)	0.314	0.546 (43)	0.116	1.000 (50)	1.000 (50)	-	
	Aug.	21	0.655 (74)	0.667 (74)	0.615 (74)	1.000 (74)	-	0.466	0.466 (74)	0.048	1.000 (74)	1.000 (74)	-	
	Aug.	22	0.809 (76)	0.808 (75)	0.767 (75)	0.991 (74)	0.905 (58)	0.422	0.382 (51)	0.167	1.000 (73)	1.000 (73)	-	
	Oct.	22	0.740 (50)	0.917 (50)	0.830 (50)	1.000 (50)	0.960 (50)	0.360	0.560 (50)	0.060	1.000 (50)	1.000 (50)	-	
	Dec.	22	0.724 (49)	0.878 (49)	0.780 (49)	1.000 (49)	-	0.512	0.433 (45)	0.022	1.000 (49)	1.000 (45)	-	
	Aug.	23	0.912 (17)	0.882 (17)	0.794 (17)	1.000 (17)	-	0.529	0.441 (17)	0.058	1.000 (17)	1.000 (17)	-	
	Oct.	23	0.770 (50)	0.924 (33)	0.724 (49)	0.990 (47)	0.930 (50)	0.511	0.405 (47)	0.042	1.000 (50)	1.000 (47)	-	
	Dec.	23	0.830 (50)	0.750 (50)	0.680 (50)	1.000 (50)	-	0.300	0.250 (20)	0.250	1.000 (49)	1.000 (48)	-	

B. (*Continued*)

Year	Time of sampling	Site number	LDH-B2*	PGM-1*	PGM-2*	GPI-B*	mAAT-1*	ALAT*			MDH-B1,2*	PEP-LT2*	PEP-C*
			*100	*null	*100	*100	*100	*100	*91	*95	*100	*100	*100
	Aug.	24	0.840	0.844	0.685	1.000	0.979	0.446	0.432	0.095	1.000	1.000	-
			(47)	(45)	(46)	(48)	(48)		(37)		(48)	(47)	-
	Oct.	24	0.929	0.714	0.698	1.000	0.920	0.542	0.385	0.063	1.000	1.000	-
			(49)	(49)	(48)	(48)	(50)		(48)		(50)	(48)	-
	Aug.	25	0.759	0.842	0.737	1.000	-	0.444	0.537	0.000	1.000	-	-
			(27)	(38)	(38)	(38)	-		(38)		(38)	-	-
	Aug.	26	0.853	0.872	0.695	1.000	-	0.392	0.549	0.029	1.000	-	-
			(51)	(39)	(59)	(59)	-		(59)		(58)	-	-
	Aug.	27	0.787	0.867	0.672	1.000	0.983	0.459	0.434	0.041	1.000	1.000	-
			(61)	(60)	(61)	(60)	(31)		(61)		(61)	(60)	-
	Aug.	28	0.812	0.827	0.698	0.994	0.967	0.358	0.534	0.097	0.994	1.000	-
			(109)	(52)	(111)	(80)	(90)		(88)		(109)	(100)	-
	Dec.	28	0.780	0.770	0.770	1.000	1.000	0.521	0.406	0.042	1.000	1.000	-
			(50)	(50)	(50)	(50)	(50)		(48)		(50)	(50)	-
	Sep.°	29	0.833	0.833	0.677	1.000	0.877	0.457	0.425	0.118	1.000	1.000	-
			(48)	(48)	(48)	(47)	(49)		(47)		(48)	(48)	-
	Dec.	30	0.820	0.750	0.690	0.990	-	0.443	0.459	0.041	0.990	1.000	-
			(50)	(50)	(50)	(50)	-		(50)		(50)	(50)	-
1990	July	17	0.774	0.846	0.720	0.969	0.927	0.377	0.469	0.016	1.000	0.989	0.889
			(93)	(94)	(96)	(48)	(96)		(65)		(48)	(48)	(19)
	Aug.	17	0.867	0.908	0.755	1.000	0.939	0.294	0.647	0.044	1.000	1.000	-
			(49)	(49)	(50)	(49)	(49)		(34)		(49)	(49)	-
	Aug.	18	0.846	0.857	0.760	1.000	0.949	0.359	0.526	0.090	1.000	1.000	0.832
			(39)	(49)	(48)	(49)	(39)		(39)		(39)	(39)	(39)
	Aug.	19	0.790	0.789	0.730	1.000	0.970	0.580	0.360	0.060	1.000	1.000	-
			(50)	(45)	(50)	(50)	(49)		(50)		(50)	(50)	-
	Oct.	19	0.740	0.880	0.687	1.000	0.930	0.344	0.625	0.000	1.000	1.000	0.800
			(48)	(46)	(48)	(50)	(50)		(48)		(48)	(48)	(45)
	Aug.	20	0.880	0.860	0.650	1.000	1.000	0.512	0.451	0.012	1.000	1.000	0.836
			(50)	(43)	(50)	(50)	(47)		(41)		(43)	(43)	(50)
	Oct.	20	0.830	0.950	0.740	1.000	0.900	0.300	0.700	0.000	1.000	1.000	0.958
			(50)	(50)	(50)	(49)	(50)		(50)		(50)	(50)	(49)
	Sep.	21	0.830	0.820	0.650	1.000	0.960	0.310	0.650	0.020	1.000	1.000	-
			(50)	(49)	(50)	(46)	(45)		(50)		(50)	(50)	-
	July	22	0.720	0.844	0.630	1.000	0.888	0.460	0.440	0.040	1.000	1.000	0.800
			(50)	(45)	(50)	(49)	(49)		(50)		(50)	(50)	(50)
	Sep.	22	0.786	0.756	0.720	1.000	0.957	0.310	0.670	0.000	1.000	1.000	0.768
			(50)	(45)	(50)	(50)	(47)		(50)		(50)	(50)	(48)
	Oct.	23	0.860	0.922	0.745	0.990	0.956	0.333	0.625	0.032	0.980	1.000	0.922
			(50)	(45)	(49)	(49)	(45)		(48)		(49)	(49)	(45)
	Oct.	24	0.780	0.831	0.720	1.000	0.934	0.500	0.459	0.031	1.000	1.000	0.796
			(48)	(48)	(50)	(43)	(48)		(43)		(43)	(43)	(43)
	Oct.	26	0.850	0.750	0.800	1.000	0.941	0.400	0.575	0.025	1.000	1.000	0.860
			(50)	(50)	(50)	(50)	(50)		(50)		(50)	(50)	(50)
	Sep.	28	0.790	0.957	0.740	1.000	0.970	0.460	0.520	0.000	1.000	1.000	0.920
			(50)	(47)	(50)	(50)	(50)		(50)		(50)	(50)	(50)
	Oct.	29	0.880	0.860	0.790	1.000	0.940	0.420	0.540	0.010	1.000	1.000	-
			(50)	(50)	(50)	(50)	(50)		(50)		(50)	(50)	-
	Oct.	30	0.830	0.910	0.730	1.000	0.960	0.396	0.511	0.031	0.980	1.000	0.865
			(50)	(50)	(50)	(50)	(50)		(48)		(49)	(49)	(48)
	July	31	0.766	0.802	0.641	1.000	0.953	0.471	0.490	0.039	1.000	1.000	0.890
			(49)	(53)	(53)	(53)	(53)		(51)		(53)	(53)	(50)
	Oct.	32	0.830	0.812	0.710	1.000	0.945	0.598	0.391	0.011	1.000	0.980	-
			(50)	(50)	(50)	(49)	(50)		(46)		(44)	(44)	-
Lake Clark													
1988		36	0.830	0.632	0.830	1.000	0.830	0.760	0.130	0.110	1.000	1.000	-
			(50)	(50)	(50)	(50)	(50)		(50)		(50)	(50)	-
		37	0.866	0.721	0.790	1.000	0.940	0.704	0.184	0.112	1.000	1.000	-
			(50)	(50)	(50)	(50)	(50)		(49)		(50)	(50)	-
		38	0.860	0.707	0.730	1.000	0.920	0.600	0.300	0.100	1.000	1.000	-
			(50)	(50)	(50)	(50)	(50)		(50)		(50)	(50)	-
Iliamna Lake													
1989		42	0.880	0.883	0.806	1.000	0.917	0.480	0.449	0.071	0.990	1.000	-
			(50)	(50)	(50)	(50)	(48)		(49)		(50)	(50)	-
		43	0.910	0.849	0.740	1.000	0.950	0.430	0.530	0.040	1.000	1.000	-
			(50)	(50)	(50)	(50)	(50)		(50)		(50)	(50)	-
		45	0.950	0.812	0.760	1.000	0.936	0.490	0.500	0.010	1.000	1.000	-
			(50)	(50)	(50)	(50)	(39)		(50)		(50)	(50)	-
		46	0.900	0.833	0.796	1.000	0.906	0.398	0.571	0.011	1.000	1.000	-
			(50)	(49)	(49)	(50)	(48)		(49)		(50)	(49)	-
		47	0.870	0.872	0.760	1.000	0.911	0.380	0.420	0.120	1.000	1.000	-
			(50)	(50)	(50)	(50)	(45)		(50)		(50)	(50)	-
1991		39	0.949	0.806	0.837	1.000	0.939	0.490	0.429	0.082	1.000	1.000	1.000
			(49)	(49)	(49)	(49)	(49)		(49)		(49)	(49)	(49)
		40	0.900	0.800	0.816	1.000	1.000	0.430	0.490	0.070	1.000	1.000	0.990
			(50)	(50)	(49)	(49)	(50)		(50)		(50)	(50)	(50)
		41	0.931	0.794	0.843	1.000	0.959	0.382	0.520	0.098	1.000	1.000	1.000
			(51)	(51)	(51)	(51)	(49)		(51)		(51)	(51)	(51)
		43	0.873	0.853	0.745	1.000	0.971	0.284	0.637	0.078	1.000	1.000	1.000
			(51)	(51)	(51)	(51)	(51)		(51)		(51)	(51)	(51)
		44	0.898	0.833	0.816	1.000	0.895	0.373	0.568	0.059	1.000	1.000	1.000
			(59)	(57)	(57)	(57)	(57)		(50)		(50)	(50)	(50)
		45	0.907	0.840	0.764	1.000	0.962	0.370	0.583	0.046	1.000	1.000	0.943
			(54)	(53)	(53)	(53)	(53)		(54)		(54)	(54)	(54)
Naviadin Lake													
1982		54°	0.434	0.826	0.955	1.000	-	-	-	-	1.000	-	-
			(99)	(92)	(99)	(98)	-	-	-	-	(99)	-	-
1984		54°	0.430	0.890	0.935	1.000	-	-	-	-	1.000	-	-
			(100)	(100)	(100)	(100)	-	-	-	-	(100)	-	-
1986		54	0.490	0.910	0.970	1.000	-	0.337	0.584	0.042	1.000	-	0.995
			(99)	(100)	(100)	(100)	-		(95)		(100)	-	(100)
		55	0.319	0.880	0.941	1.000	-	0.338	0.574	0.016	1.000	-	1.000
			(94)	(94)	(94)	(93)	-		(94)		(94)	-	(94)
		56	0.394	0.914	0.975	1.000	-	0.337	0.597	0.046	1.000	-	0.990
			(99)	(99)	(99)	(94)	-		(98)		(99)	-	(99)
1987		54°	0.475	0.840	0.970	1.000	-	0.325	0.655	0.020	1.000	-	-
			(100)	(100)	(100)	(100)	-		(97)		(100)	-	-
Rabine Lake													
1985		57	0.953	0.793	0.766	-	-	0.388	0.518	0.088	-	-	0.989
			(96)	(94)	(94)	-	-		(85)		-	-	(93)
		59	0.971	0.786	0.838	-	-		-		-	-	-
			(103)	(103)	(103)	-	-		-		-	-	-
		60	0.977	0.801	0.805	-	-		-		-	-	-
			(150)	(146)	(146)	-	-		-		-	-	-
		63	0.975	0.768	0.779	-	-		-		-	-	-
			(100)	(95)	(95)	-	-		-		-	-	-
1987		57	0.944	0.839	0.753	1.000	-	0.466	0.472	0.063	1.000	-	-
			(89)	(87)	(89)	(82)	-		(88)		(89)	-	-
		58	0.975	0.810	0.780	1.000	-	0.489	0.426	0.085	1.000	-	-
			(100)	(100)	(100)	(98)	-		(94)		(100)	-	-
		59	0.985	0.805	0.735	1.000	-	0.458	0.463	0.079	1.000	-	-
			(100)	(100)	(100)	(91)	-		(95)		(99)	-	-
		60	0.975	0.850	0.765	1.000	-	0.413	0.418	0.168	1.000	-	-
			(100)	(100)	(100)	(94)	-		(98)		(99)	-	-
		61	0.987	0.776	0.744	1.000	-	0.461	0.494	0.045	1.000	-	-
			(78)	(78)	(78)	(75)	-		(77)		(78)	-	-
		63	0.980	0.780	0.750	1.000	-	0.333	0.606	0.056	1.000	-	-
			(100)	(100)	(100)	(100)	-		(99)		(100)	-	-
		64	0.975	0.842	0.753	-	-	0.449	0.513	0.038	-	-	-
			(79)	(79)	(79)	-	-		(79)		-	-	-

B. (Concluded)

Year	Time of[a] sampling	Site number	*LDH-B2*[a] *100	*PGM-1*[a] *null	*PGM-3*[a] *100	*GPI-B*[a] *100	*mAAT-1*[a] *100	*ALAT*[a] *100	*91	*93	*MDH-B1,3*[a] *100	*PEP-LT2*[a] *100	*PEP-C*[a] *100
		65	0.985	0.760	0.808	1.000	-	0.476	0.457	0.061	1.000	-	-
			(99)	(98)	(98)	(98)	-		(82)		(99)	-	-
		66	0.954	0.852	0.791	1.000	-	0.436	0.457	0.085	1.000	-	-
			(98)	(98)	(98)	(98)	-		(94)		(98)	-	-
1988		57	0.960	0.773	0.760	1.000	-	0.447	0.427	0.120	1.000	-	0.973
			(75)	(75)	(75)	(75)	-		(75)		(75)	-	(74)
		58	0.975	0.797	0.766	1.000	-	0.487	0.437	0.076	1.000	-	0.886
			(79)	(79)	(79)	(79)	-		(79)		(79)	-	(79)
		59	0.970	0.810	0.780	1.000	-	0.445	0.490	0.065	1.000	-	0.910
			(100)	(100)	(100)	(100)	-		(100)		(99)	-	(100)
		62	0.974	0.836	0.809	1.000	-	0.420	0.467	0.100	1.000	-	0.934
			(76)	(76)	(76)	(76)	-		(75)		(76)	-	(76)
		64	0.982	0.871	0.777	-	-	0.488	0.441	0.071	1.000	-	0.906
			(85)	(85)	(85)	-	-		(85)		(85)	-	(85)
1990		60	0.974	0.822	0.789	1.000	0.951	0.397	0.518	0.062	1.000	0.986	-
			(499)	(499)	(497)	(499)	(499)		(505)		(499)	(367)	-
		63	0.970	0.810	0.751	1.000	0.971	0.346	0.588	0.050	1.000	0.969	-
			(399)	(400)	(400)	(400)	(400)		(399)		(400)	(370)	-
Shuswap Lake													
1983		67	0.995	0.586	0.842	-	-[c]	0.786	0.149	0.039	-	-	1.000
			(97)	(99)	(101)	-	-		(77)		-	-	(81)
1986		67	0.990	0.417	0.870	0.997	-	0.688	0.133	0.115	0.975	-	1.000
			(146)	(144)	(142)	(146)	-		(144)		(142)	-	(141)
1990		67	1.000	0.393	0.918	1.000	-	0.626	0.136	0.116	1.000	-	1.000
			(94)	(98)	(98)	(99)	-		(99)		(99)	-	(99)
		68	0.995	0.265	0.835	0.995	-	0.735	0.195	0.055	0.930	-	1.000
			(100)	(100)	(100)	(100)	-		(100)		(100)	-	(100)

[a] all sites were sampled during spawning activity (except site 54 as noted); the month of sampling is indicated if sites were sampled more than once; the number of fish sampled for each collection is in parentheses.
[b] "-" means no data were collected for this locus.
[c] late September, considered as a "later" spawning site.
[d] sampled at inlet to lake except in 1986; fish had not yet segregated by spawning site.

Electrophoretic Characterization of Odd-Year Pink Salmon (*Oncorhynchus gorbuscha*) Populations from the Pacific Coast of Russia, and Comparison with Selected North American Populations

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We collected and electrophoretically analyzed a total of 558 fish from eight locations along the Pacific Coast of Russia. We successfully screened 44 enzyme-coding loci: 14 loci were polymorphic at the 0.95 level in at least one collection, an additional eight were polymorphic at the 0.99 level but not at the 0.95 level, and the remaining 22 were either monomorphic or exhibited only very rare variation in these collections. Contingency χ^2 tests using the 23 most variable loci revealed significant heterogeneity among all eight collections ($p = 0.028$) but little or no significant heterogeneity among collections within areas (northeastern Kamchatka peninsula, $p = 0.180$; southwestern Kamchatka, $p = 0.533$; and mainland adjacent to the northwestern Sea of Okhotsk, $p = 0.071$). Multidimensional scaling and minimum spanning tree analyses using genetic distances among collections indicated that geographic proximity of spawning sites was not associated with genetic similarity. The eight odd-year pink salmon (*Oncorhynchus gorbuscha*) collections from Russia were compared with 16 collections from North America (southeastern Alaska, British Columbia, and Washington) using data for 33 loci. The Russian populations differed from the North American populations in their patterns of allelic variation at many loci. The amount of genetic differentiation among populations from different rivers in Russia was comparable to that seen within similar-sized areas in North America.

Les auteurs ont prélevé un total de 558 saumons roses à huit endroits de la côte russe du Pacifique, et les ont soumis à une analyse électrophorétique. Ils ont réussi à identifier 44 loci codeurs d'enzymes : 14 étaient polymorphes à un niveau de confiance de 95 % dans au moins une collection, 8 autres étaient polymorphes à un niveau de confiance de 99 % mais non de 95 %, et les 22 autres étaient soit monomorphes ou ne variaient que rarement dans ces collections. Des tests de contingence de χ^2 portant sur les 23 loci les plus variables ont révélé un hétérogénéité significative entre les huit collections ($p = 0,028$), mais peu ou pas d'hétérogénéité significative entre les collections intra-régionales (nord-est de la péninsule de Kamchatka, $p = 0,180$; sud-ouest de la péninsule de Kamchatka, $p = 0,533$; et côte continentale du nord-ouest de la mer d'Okhotsk, $p = 0,071$). Une analyse multidimensionnelle et des analyses de l'arbre du poids minimum basées sur la distance au plan génétique entre les collections ont révélé l'absence d'association entre la proximité géographique des frayères et la similitude génétique. En utilisant les données sur 33 loci, les auteurs ont comparé les huit collections de saumons roses (*Oncorhynchus gorbuscha*) obtenues en Russie pendant des années de faible montaison et 16 collections provenant de l'Amérique du Nord (sud-est de l'Alaska, Colombie-Britannique et État de Washington). Les populations russes se distinguaient des populations nord-américaines par leur régime de variation allélique à de nombreux loci. Le niveau de différenciation génétique des populations de différents cours d'eau de la Russie se compare à celui observé dans certaines régions de l'Amérique du Nord de superficie semblable.

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Successful long-term fisheries management and conservation require knowledge of genetic aspects of stock structure of the species in question. This need is especially acute for Pacific salmon because they exist in numerous more or less reproductively independent population units that have resulted from the strong homing behavior these species exhibit, their anadromous life cycles, and the extensive geographic ranges they occupy (Shaklee et al. 1990b; 1990c).

Published electrophoretic analyses of pink salmon (Aspinwall 1974; Altukov et al. 1983; Beacham et al. 1985a, 1988; Gharrett et al. 1988; Shaklee et al. 1991), chum salmon

(*Oncorhynchus keta*) (Okazaki 1982; Beacham et al. 1985b; Phelps et al. 1994), and chinook salmon (*O. tshawytscha*) (Gharrett et al. 1987; Utter et al. 1989; Bartley and Gall 1990) document both the number and diversity of genetically distinctive population units in these species.

Pink salmon (*O. gorbuscha*) are unique among Pacific salmon because they exhibit a rigid 2-yr life cycle (Davidson 1934). This life history has led to the differentiation of two distinct races of pink salmon (odd and even year) that exhibit pronounced genetic divergence, even between populations spawning at the same locations in alternate years (Aspinwall 1974; McGregor 1983; Beacham et al. 1985a, 1988; Gharrett and Smoker 1991). Shaklee et al. (1991) have recently increased the number of loci and alleles recognized as varying in odd-year pink salmon from Washington and British Columbia, have genetically characterized 26 stocks in these areas, and have described the genetic interrelationships of these stocks. Their analysis showed a clear geographic pattern to stock relationships throughout this portion of the species' range.

We undertook the present investigation to determine the pattern of stock interrelationships among odd-year pink salmon populations along the Pacific Coast of Russia and to compare the pattern and amount of genetic variation exhibited by these Russian pink salmon with those found in Washington and British Columbia.

Materials and Methods

Samples of skeletal muscle, heart, eye, and liver were obtained from adult pink salmon collected on their natural spawning grounds or during their upstream migrations in eight different river systgems along the Russian Pacific Coast (Fig. 1 and Table 1). Because logistical constraints prevented sampling each population throughout the spawning run, each population was sampled on a single day (Table 1). Approximately 1-g samples of each tissue were dissected and placed in small, individually labeled polyethylene containers and frozen in liquid nitrogen in the field. The samples were stored in liquid nitrogen for approximately 1 yr before they were electrophoretically analyzed. Because some samples thawed briefly at least once during storage, tissue quality at the time of electrophoretic analysis (July 1992) was judged as only fair.

Extracts of muscle, eye, and liver were prepared by gently hand homogenizing one volume of tissue using a loose-fitting metal rod in a plastic culture tube with an equal volume of TC-1 gel buffer (Shaw and Prasad 1970) containing 5 mg/mL pyridoxal 5′-phosphate. The extracts were then centrifuged for 5 min at approximately 1000 × *g*. Portions of the resulting supernatants were absorbed in 4 × 8 mm chromatography paper wicks (Schleicher and Schuell No. 470) and loaded directly on horizontal starch gels made using Connaught starch. We used standard procedures of horizontal starch-gel electrophoresis and histochemical staining as described by Shaklee and Keenan (1986), Aebersold et al. (1987), and Morizot and Schmidt (1990). Our electrophoretic screening protocol included the analysis of many loci two or more times using different buffers and/or tissues to ensure the accuracy of isozyme pattern interpretation and data collection (Shaklee and Phelps 1990). Individual scores that were ambiguous were not used. Loci where fewer than 90% of the individuals in a collection were reliably

FIG. 1. Map of Russian sampling localities.

scored were similarly excluded (except for *sAH** and *GPI-A** in the Kik-Chik River collection). The specific electrophoretic conditions used, loci screened, and alleles scored are listed in Table 2. All electrophoretic phenotypes were independently scored by two individuals who entered the data (presumed genotypes) directly in computer files using a computer program that automatically compared the scores to identify any discrepancies. Locus and allele nomenclature follow Shaklee et al. (1990a).

In a few cases, rare alleles were pooled to make the data set consistent with that for the Washington and British Columbia collections (Shaklee et al. 1991; White and Shaklee 1991) or to eliminate ambiguity in allele identification. This was done for the *ALAT*108* and **106* alleles (pooled with the *ALAT*111* allele), the *GDA*117*, **115*, and **123* alleles (pooled with the *GDA*113* allele), and the *PEPD-2*110* and **130* alleles (pooled with the *PEPD*120* allele).

Statistical analyses of the resulting data were performed using the BIOSYS-1 computer program of Swofford and Selander (1981), a *G*-test program written by R. Waples (NMFS, Seattle, WA), and the NTSYS-pc computer program of Rohlf (1992).

TABLE 1. Information regarding the 23 collections of odd-year and one collection of even-year pink salmon analyzed in the present study.

Region and location	Collection date	Sample size
Chukotka Peninsula		
Anadyr River	29 July 1991	80
Kamchatka Peninsula, northeast coast		
Uilali-Vaiam River	6 Aug. 1991	79
Ivashka River	26 July 1991	80
Kamchatka Peninsula, southwest coast		
Kik-Chik River	29 July 1991	40
Pymta River	1 Aug. 1991	79
Kol River	31 July 1991	40
Okhotsk Sea, northwest coast		
Tumani River	17 July 1991	80
Arman River	15 July 1991	80
Southeastern Alaska		
Port Caldera Creek	21 Sept. 1987	50
Fish Creek	26 Aug. 1987	50
Naha River	14 Aug. 1987	50
Hugh Smith River	6 Sept. 1987	50
British Columbia		
Babine River	14 Sept.1991	100
Atnarko River	22 Oct. 1991	100
Kakweiken River	5 Sept. 1991	101
Puntledge Hatchery	4 Oct. 1991	100
Thompson River	16, 17 Oct. 1991	100
Seton Creek	22 Oct. 1991	100
Washington		
middle fork Nooksack River	18 and 25 Sept. 1991	100
Skagit River	2–17 Oct. 1991	100
Snohomish River	30 Sept. and 8 Oct. 1991	100
Dosewallips River	27 Sept. and 1 Oct. 1991	100
Nisqually River	3–16 Oct. 1991	100
Snohomish River [even-year]	14–24 Sept. 1990	75

Results

All samples were analyzed using the same basic screening protocols of Shaklee et al. (1991), except that heart tissue was not analyzed; FH, LGL, PGK, and TPI were not stained for; and ENO and NTP were added to the screening protocol. The condition of the Russian samples allowed us to collect reliable data for only 44 loci. Unfortunately, we were not able to obtain reliable scores for several loci (*mAH-4**; *CK-C1**; *CK-C2**; and *sIDHP-2**) known to vary in North American pink salmon stocks (Shaklee et al. 1991).

Nine loci were monomorphic for the **100* allele in all fish at all locations (*AK**; *CK-A2**; *ENO-1**; *mIDHP-2**; *LDH-A1**; *LDH-B1**; *LDH-B2**; *NTP**; and *PEPA**). Three loci were monomorphic in all the fish at all the locations we screened successfully, but were not screened successfully at one location each (*CK-B** from Anadyr River; *MPI** from Ivashka River; and *sSOD-2** from Anadyr River). The remaining 32 loci were variable in one or more of the eight Russian collections, and the allele frequencies for these loci at each site are presented in Table 3. Fourteen loci (*sAAT-3**; *sAAT-4**; *ADA-2**; *FDHG**; *GDA**; *G3PDH-1**; *sMDH-B1,2**; *mMEP-1**; *PEPB-1**; *PEPD-2**; *PEP-LT**; *PGDH**; and *PGM-2**) were polymorphic at the $P_{0.95}$ level in at least one collection. An additional eight loci (*mAAT-1**; *sAH**; *ALAT**; *GPI-A**; *GR**; *sMDH-A1,2**; and *mMEP-2**) were polymorphic at the $P_{0.99}$ level in at least one collection. The observed average heterozygosity per collection (using all 44 loci successfully screened) ranged from 0.058 in the Kik-Chik River sample to 0.075 in the Ivashka River sample.

We tested the allele counts at all variable loci for departures from Hardy–Weinberg expectations. There were 110 tests where the number of observed variants at a locus in a collection exceeded five. Of these, there were five statistically significant ($p < 0.05$) deviations where two or more of the sampled fish had improbable genotypes (*sAAT-3** in the Arman River, *mMEP-1** in the Tumani River, *PEPD-2** in both the Uilali-Vaiam and Kol rivers, and *PGDH** in the Kol River. Because this number of significant deviations is approximately the number expected from performing 110 tests at $\alpha = 0.05$, and because only one locus exhibited more than one significant deviation (*PEPD-2** had two), we conclude that all the populations were in Hardy–Weinberg equilibrium and that there is no clear indication that any of the individual collections were derived from more than a single gene pool.

We conducted contingency χ^2-tests to examine genetic interrelationships among the eight collections. A simultaneous χ^2-test using all eight collections revealed significant heterogeneity at three loci (*ADA-2**, $p = 0.013$; *PEPD-2**,

TABLE 2. Locus designations, recognized alleles, tissue source(s), and screening buffer(s) for pink salmon analyzed in the present study.

Locus[a]	Alleles a	b	c	d	e	f	g	h	i	Tissue	Buffer(s)[b]
mAAT-1	−100	−83	−70	−108						M	CAME6.8
mAAT-2[c]	−100	−69	−109							M	CAME6.8
sAAT-1,2	100	110	61	83						H	CAME6.8
sAAT-3	100	91	79							E	**LIOH-RW**; TRIS-GLY; TECB
sAAT-4	100	190	268	18						L	**TRIS-GLY** & CAM6.8
ADA-1	100	86	122							M	CAM6.1
ADA-2	100	110	90[d]	XXX[d]	114					M,E	**CAM 6.1**; [CAME6.8]
sAH	100	115	90							L	CAM6.8
AK	−100	−145								M	TRIS-GLY
ALAT	100	111	108	88	78	106				M	**CAME6.8**; TRIS-GLY
CK-A1	100	66	110	74						M	TRIS-GLY
CK-A2	100	108	82	119	90					M	TRIS-GLY
ENO-1	−100									M	CAME6.8
FDHG	100	138	58							L,M	TRIS-GLY
GDA	100	108	113		118	115	123	82	110	L	CAM(E)6.8 & **TRIS-GLY**
GPI-A	100	108	91	88	120					M	TRIS-GLY
GPI-B1,2	100	200	−64	25	229					M	TRIS-GLY
GR	100	109	88							E,L	**TECB**; LIOH-RW
G3PDH-1	−100	−170	60	−10	20	200				M	**CAME6.8** & TRIS-GLY
mIDHP-1	100	165	45	18						M	CAME6.8
mIDHP-2	100	118								M	CAME6.8
LDH-A1	−100	−250								M	TRIS-GLY
LDH-A2	100									M	TRIS-GLY
LDH-B1	100	192	86							M,E	**TRIS-GLY**; TC-4
LDH-B2	100	132	74[e]							L[e] (E)	TRIS-GLY
LDH-C	100	90	112							E	**TECB**; TRIS-GLY
sMDH-A1,2	100	152	−35	40						L,E,M	**TC-4**; CAM6.1; CAME6.8
sMDH-B1,2	100	121	70			85[f]	130	44		M	**TC-4** & **CAME6.8**
mMEP-1[g]	100	123	115							M	CAME6.8
mMEP-2[g]	100	70	123							M	CAME6.8
MPI	100	105	85							E	**TRIS-GLY**; EBT
NTP	100	53								M,L	**CAME6.8**; LIOH-RW
PEPA	100	109								M	TRIS-GLY
PEPB-1	100	138	50	200						M,L	**LIOH-RW**; TRIS-GLY
PEPD-2	100	120	80	110	130					M,L	**CAME6.8**; TRIS-GLY
PEP-LT	100	108	90	80						M(L)	TC-4; **TRIS-GLY**
PGDH	100	108	94	90						M,E	CAM(E)6.8; **TC-4**
PGM-2	100	155	135	25	250					M,E	TRIS-GLY
sSOD-1	100	245								L,M	CAM6.1; **TRIS-GLY**
sSOD-2[h]	100	122								M	**CAM6.1**; TC-4; TRIS-GLY

[a]Equivalences with previously used locus designations: *AAT-3 = Aat; ADA-2 = Ada; CK-A1 = Ck-1; FDHG = HAGH; GPI-A = Pgi-3; GPI-B1,2 = Pgi-1,2; G3PDH-1 = Agp; sIDHP-2 = Idh-3; LDH-A1 = Ldh-1; LDH-B1 = Ldh-3; LDH-B2 = Ldh-4; sMDH-A1,2 = Mdh-1,2; sMDH-B1,2 = Mdh-3,4; mMEP-1 = Me; PEP-B1 = Lgg; PEP-D2 = Pp; PGDH = 6Pg*

[b]Best buffer listed first; allele designations based on isozyme mobilities using buffer in **bold type**

[c]Variation at this locus is scored from the banding patterns of the mAAT-1/mAAT-2 interlocus heterodimer

[d]The *ADA-2*90* allele is scored best on CAM6.1 and cannot be scored reliably on TC-4; the **d* (=**105*) allele could not be screened in these samples because this locus was not screened on TC-4; the **110* allele is scored best on CAM6.1 (and is not scored reliably on CAME6.8)

[e]The *LDH-B2*74* allele can only be scored reliably in liver tissue (where *LDH-B1** is not expressed)

[f]The *sMDH-B1,2*85* allele is reliably resolved only on TC-4 & CAM6.1 (or other low pH buffers)

[g]The **100* alleles at *mMEP-1** and *mMEP-2** co-migrate

[h]Variation at this locus is scored from the banding patterns of the sSOD-1/sSOD-2 interlocus heterodimer

– allele not observed previously by JBS (Shaklee et al. 1991 & unpublished data) in pink salmon from North America (primarily Washington and southern British Columbia stocks).

$p = 0.009$; and *PEP-LT**, $p = 0.005$). While the total χ^2-value from this test was nonsignificant using all 32 variable loci, it was significant ($p = 0.028$) when the test was restricted to only those loci polymorphic at the $P_{0.99}$ level.

Having detected some heterogeneity among the eight collections with the latter test, we then conducted χ^2-tests among collections within the three regions represented by our samples: northeastern Kamchatka (the Uilali-Vaiam and Ivashka rivers), southwestern Kamchatka (the Kik-Chik, Pymta, and Kol rivers), and the northwestern coast of the Okhotsk Sea (the Tumani and Arman rivers). For the northeastern Kamchatka region, no individual loci exhibited

TABLE 3. Allele frequencies at 32 variable loci in odd-year pink salmon from eight rivers in the Kamchatka region of Russia. (*N*) = number of fish successfully screened in each collection.

	Population							
Locus	Anadyr	Uilali–Vaiam	Ivashka	Kik–Chik	Pymta	Kol	Tumani	Arman
mAAT-1								
**-100*	0.994	0.994	1.000	0.988	0.981	1.000	0.973	1.000
**-108*	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000
**-83*	0.006	0.006	0.000	0.013	0.019	0.000	0.020	0.000
(*N*)	(79)	(79)	(80)	(40)	(79)	(40)	(75)	(80)
mAAT-2								
**-100*	1.000	1.000	0.994	1.000	0.994	1.000	1.000	1.000
**-109*	0.000	0.000	0.006	0.000	0.006	0.000	0.000	0.000
(*N*)	(79)	(79)	(80)	(40)	(79)	(40)	(76)	(80)
sAAT-1,2								
**100*	1.000	1.000	1.000	1.000	0.994	1.000	0.994	1.000
**83*	0.000	0.000	0.000	0.000	0.006	0.000	0.006	0.000
(*N*)	(80)	(79)	(80)	(40)	(79)	(40)	(78)	(80)
sAAT-3								
**100*	0.993	0.905	0.951	0.931	0.934	0.949	0.955	0.899
**91*	0.007	0.088	0.049	0.056	0.066	0.051	0.045	0.095
**79*	0.000	0.007	0.000	0.014	0.000	0.000	0.000	0.006
(*N*)	(72)	(74)	(72)	(36)	(76)	(39)	(56)	(79)
sAAT-4								
**100*	0.400	0.468	0.367	0.311	0.377	0.372	0.418	0.408
**190*	0.600	0.532	0.633	0.689	0.623	0.628	0.575	0.592
**18*	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000
(*N*)	(65)	(79)	(79)	(37)	(73)	(39)	(73)	(76)
ADA-1								
**100*	1.000	1.000	0.994	1.000	1.000	1.000	0.994	1.000
**122*	0.000	0.000	0.006	0.000	0.000	0.000	0.006	0.000
(*N*)	(80)	(79)	(80)	(40)	(79)	(40)	(79)	(80)
ADA-2								
**100*	0.943	0.899	0.900	0.925	0.930	0.913	0.877	0.906
**110*	0.000	0.000	0.019	0.038	0.000	0.000	0.000	0.006
**90*	0.057	0.101	0.081	0.038	0.070	0.087	0.123	0.087
(*N*)	(79)	(79)	(80)	(40)	(79)	(40)	(73)	(80)
sAH								
**100*	0.967	0.981	0.987	0.971	0.994	0.962	0.994	0.987
**115*	0.027	0.019	0.006	0.029	0.006	0.038	0.006	0.007
**90*	0.007	0.000	0.006	0.000	0.000	0.000	0.000	0.007
(*N*)	(75)	(79)	(79)	(35)	(78)	(40)	(78)	(76)
ALAT								
**100*	0.988	1.000	0.981	1.000	0.981	1.000	1.000	0.988
**111+*	0.013	0.000	0.019	0.000	0.019	0.000	0.000	0.013
(N)	(80)	(79)	(79)	(40)	(79)	(40)	(77)	(80)
CK-A1								
**100*	0.994	1.000	0.994	1.000	0.994	1.000	1.000	1.000
**110*	0.006	0.000	0.006	0.000	0.006	0.000	0.000	0.000
(*N*)	(79)	(79)	(80)	(40)	(79)	(40)	(80)	(80)
FDHG								
**100*	0.925	0.956	0.950	0.938	0.949	0.938	0.968	0.944
**138*	0.075	0.044	0.050	0.063	0.051	0.063	0.032	0.056
(*N*)	(80)	(79)	(80)	(40)	(79)	(40)	(79)	(80)

TABLE 3. (*Continued*)

Locus	Population							
	Anadyr	Uilali–Vaiam	Ivashka	Kik–Chik	Pymta	Kol	Tumani	Arman
GDA								
**100*	0.584	0.532	0.586	0.625	0.608	0.603	0.527	0.544
**113*	0.097	0.154	0.099	0.100	0.108	0.128	0.158	0.118
**108*	0.318	0.314	0.316	0.275	0.284	0.269	0.315	0.338
(*N*)	(77)	(78)	(76)	(40)	(74)	(39)	(73)	(34)
GPI-A								
**100*	1.000	0.994	0.962	1.000	0.987	0.987	0.994	0.994
**108*	0.000	0.006	0.038	0.000	0.013	0.013	0.006	0.006
(*N*)	(76)	(77)	(26)	(26)	(77)	(39)	(79)	(80)
GPI-B1,2								
**100*	1.000	0.994	1.000	1.000	1.000	1.000	0.997	0.997
**200*	0.000	0.006	0.000	0.000	0.000	0.000	0.003	0.003
(*N*)	(79)	(79)	(71)	(40)	(79)	(40)	(80)	(80)
GR								
**100*	1.000	0.994	0.993	0.986	1.000	1.000	1.000	1.000
**88*	0.000	0.006	0.007	0.014	0.000	0.000	0.000	0.000
(*N*)	(71)	(79)	(75)	(37)	(79)	(18)	(70)	(70)
G3PDH-1								
**-100*	0.956	0.962	0.975	0.938	0.956	0.938	0.938	0.950
**-10*	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000
**60*	0.044	0.038	0.025	0.063	0.044	0.063	0.056	0.050
(*N*)	(80)	(79)	(80)	(40)	(79)	(40)	(80)	(80)
mIDHP-1								
**100*	0.994	0.994	1.000	1.000	0.994	1.000	0.994	1.000
**45*	0.006	0.000	0.000	0.000	0.006	0.000	0.006	0.000
**18*	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000
(*N*)	(80)	(79)	(80)	(40)	(79)	(40)	(78)	(80)
LDH-C								
**100*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.993
**112*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007
(*N*)	(73)	(75)	(78)	(36)	(78)	(40)	(69)	(75)
sMDH-A1,2								
**100*	0.988	0.981	0.962	1.000	0.987	0.988	0.981	0.981
**40*	0.000	0.006	0.006	0.000	0.000	0.000	0.000	0.000
**-35*	0.013	0.013	0.031	0.000	0.013	0.013	0.019	0.019
(*N*)	(80)	(79)	(80)	(40)	(79)	(40)	(80)	(80)
sMDH-B1,2								
**100*	0.894	0.924	0.887	0.887	0.880	0.950	0.931	0.919
**124*	0.056	0.057	0.050	0.063	0.057	0.038	0.044	0.050
**70*	0.050	0.019	0.063	0.050	0.063	0.013	0.025	0.031
(*N*)	(80)	(79)	(80)	(40)	(79)	(40)	(80)	(80)
mMEP-1								
**100*	0.875	0.880	0.894	0.875	0.899	0.925	0.911	0.875
**123*	0.125	0.120	0.106	0.125	0.101	0.075	0.089	0.125
(N)	(80)	(79)	(80)	(40)	(79)	(40)	(79)	(80)
mMEP-2								
**100*	1.000	1.000	1.000	1.000	1.000	1.000	0.988	0.994
**123*	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.006
(*N*)	(80)	(79)	(80)	(40)	(79)	(40)	(80)	(80)

TABLE 3. (*Concluded*)

Locus	Population							
	Anadyr	Uilali–Vaiam	Ivashka	Kik–Chik	Pymta	Kol	Tumani	Arman
PEPB-1								
**100*	0.974	0.943	0.981	0.988	0.962	0.938	0.931	0.962
**200*	0.000	0.006	0.000	0.000	0.013	0.013	0.000	0.000
**138*	0.026	0.044	0.006	0.013	0.013	0.038	0.069	0.019
**50*	0.000	0.006	0.013	0.000	0.013	0.013	0.000	0.019
(*N*)	(77)	(79)	(80)	(40)	(79)	(40)	(80)	(80)
PEPD-2								
**100*	0.734	0.753	0.719	0.675	0.734	0.775	0.821	0.644
**120*	0.228	0.190	0.262	0.262	0.184	0.213	0.160	0.313
**80*	0.038	0.057	0.019	0.063	0.082	0.013	0.019	0.044
(*N*)	(79)	(79)	(80)	(40)	(79)	(40)	(78)	(80)
PEP-LT								
**100*	0.746	0.842	0.825	0.865	0.753	0.775	0.857	0.775
**108*	0.021	0.026	0.044	0.014	0.063	0.038	0.013	0.044
**90*	0.232	0.132	0.132	0.122	0.184	0.188	0.130	0.156
**80*	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.025
(*N*)	(71)	(76)	(57)	(37)	(79)	(40)	(77)	(80)
PGDH								
**100*	0.835	0.880	0.869	0.925	0.861	0.813	0.913	0.925
**108*	0.000	0.006	0.006	0.000	0.000	0.000	0.000	0.000
**94*	0.133	0.108	0.125	0.063	0.114	0.125	0.063	0.063
**90*	0.032	0.006	0.000	0.013	0.025	0.063	0.025	0.013
(*N*)	(79)	(79)	(80)	(40)	(79)	(40)	(80)	(80)
PGM-2								
**100*	0.913	0.911	0.919	0.900	0.899	0.938	0.894	0.887
**155*	0.087	0.089	0.081	0.100	0.101	0.063	0.106	0.112
(*N*)	(80)	(79)	(80)	(40)	(79)	(40)	(80)	(80)
sSOD-1								
**100*	0.994	1.000	1.000	1.000	0.993	1.000	1.000	0.994
**245*	0.006	0.000	0.000	0.000	0.007	0.000	0.000	0.006
(*N*)	(80)	(79)	(80)	(40)	(74)	(40)	(80)	(80)

significant heterogeneity and the overall χ^2-value was nonsignificant. Similarly, for southwestern Kamchatka, only *ADA-2** exhibited significant heterogeneity ($p = 0.033$), and this was due to the rare occurrence of the **110* allele. When this allele was pooled (with either the **100* or the **90* allele), the test was nonsignificant. Despite the marginally significant outcome for *ADA-2**, the overall χ^2-value for this region using all loci was nonsignificant. In the northwestern coast of the Okhotsk Sea region, while both *PEPB-1** ($p = 0.022$) and *PEPD-2** ($p = 0.002$) exhibited significant heterogeneity, the overall χ^2-value using all loci was nonsignificant. Finally, we pooled the data from all the individual collections within each region and then tested among regions using the 32 polymorphic loci ($P_{0.99}$). This test yielded a significant deviation at only one locus (*PEP-LT**; $p = 0.013$) and was nonsignificant overall ($p = 0.137$).

The individual locus F_{ST} values were all small, with the highest value (0.013) observed for *sAAT-3**, *GPI-A**, and *PEPD-2**. The average F_{ST} across the eight collections and all 32 variable loci was only 0.008.

We conclude from these tests that there is slight but significant heterogeneity among the eight collections, but that there is no convincing evidence of a strong regional basis for this heterogeneity.

We computed genetic distances (using all 32 variable loci) among the eight Russian collections and used the resulting matrix to examine the relationships among the collections by multidimensional scaling. The results of this analysis, with a minimum spanning tree linking the most similar collections superimposed on the plot, are shown in Fig. 2. This graphical analysis shows the collections from the Kik-Chik, Ivashka, Arman, and Tumani rivers to be outliers. Comparison of these relationships with the locations of the rivers (Fig. 1) reveals that geographic proximity is unrelated to genetic similarity. For example, the three collections from southwestern Kamchatka (the Kik-Chik, Pymta, and Kol rivers) are not clustered together by the multidimensional scaling or linked by the minimum spanning tree. Thus, no geographic basis for the observed genetic differences was found.

We compared the eight collections of odd-year pink salmon from Russia with 15 collections of odd-year and one collection of even-year pink salmon from North America to provide a broader context in which to view the odd-year Russian pink salmon data. The collections from North America spanned a considerable portion of the species' range and included: four 1987 collections from southeastern Alaska, six 1991 collections from British Columbia, and five 1991 collections and one 1990 collection from Washington. This analysis was conducted using the 33 variable loci tabulated for the 16 North American collections in Appendix 1. We had to exclude *ADA-1** and *sSOD-1** from this analysis because we lacked data for these loci from some of the North American collections. We included *LDH-A1**; *LDH-B1**; and *LDH-B2** (which were invariant in the Russian collections) because they were variable in North America.

Figure 3 shows allele frequency variation at four of the most variable loci in the Russian and North American collections. Figure 4 shows the dendrogram that resulted from UPGMA clustering of Cavalli-Sforza and Edwards (1967) genetic distances among these 24 collections. The stock interrelationships evident here have a strong geographic component. The eight collections from Russia first cluster together and then group with a cluster of seven collections from northern North America. This latter cluster includes the four collections from southeastern Alaska, one from northern British Columbia (Babine), one from central British Columbia. (Atnarko), and one from southern British Columbia (Kakweiken). This combined cluster of 15 stocks then groups with a cluster that includes the eight remaining odd-year collections from North America: Puntledge from central Vancouver Island; Thompson and Seton from the mid-Fraser River; Nooksack, Skagit, and Snohomish from north and central Puget Sound; Dosewallips from Hood Canal; and Nisqually from south Puget Sound.

The only notable exception to the broad scale geographic patterning evident for the odd-year collections from North America is the Nooksack (from northern Puget Sound), which clusters with the collections from Hood Canal and south Puget Sound (see Discussion). The only even-year pink salmon collection included in this analysis (Snohomish 1990) was an outgroup to all the odd-year collections, even those from Russia. This result was not unexpected because the two-year life cycle of pink salmon insures essentially total reproductive isolation of the odd- and even-year races. This result is consistent with earlier published results reporting large genetic differences between even- and odd-year pink salmon (see Introduction).

Discussion

The eight Russian populations of odd-year pink salmon analyzed in this study are all wild stocks, with no history of hatchery propagation or introduction of foreign stocks. They spawn in remote areas and experience little or no impact from man's activities, except for impacts from ocean, coastal, and in-river fisheries. Thus, their genetic characteristics likely represent those that evolved in situ in this portion of the species' range.

The Russian pink salmon populations characterized in this study exhibited variation at basically the same suite of enzyme-coding gene loci as do populations from North America (Beacham et al. 1985a, 1988; Shaklee et al. 1991). However,

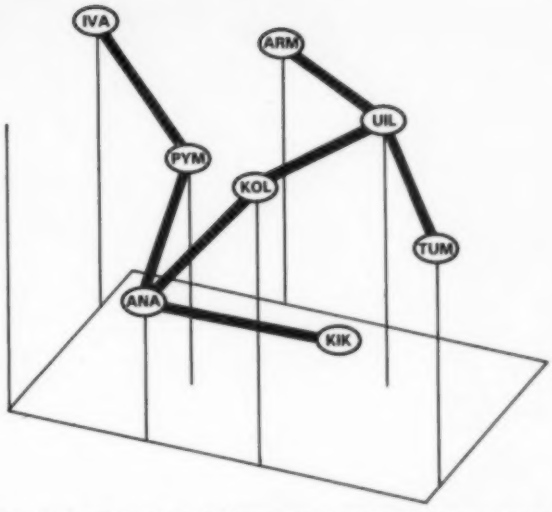

FIG. 2. Results of multidimensional scaling analysis (using Cavalli-Sforza and Edwards (1967) chord distances) with a minimum spanning tree. Collections are coded as follows: ANA = Anadyr River; UIL = Uilali-Vaiam River; IVA = Ivashka River; KIK = Kik-Chik River; PYM = Pymta River; KOL = Kol River; TUM = Tumani River; ARM = Arman River.

some loci that exhibit variation in North American stocks were monomorphic in the Russian populations (*mAAT-1**, *CK-A2**, *LDH-A1**, *LDH-B1**, *LDH-B2**, and *PEPA**). This likely occurred because all these loci (except *LDH-A1**) exhibit only rare variation in North America, and the number of fish from North America analyzed was larger than the number from Russia analyzed. The one exception (*LDH-A1**) is only rarely variable in North America, except in Washington (Shaklee et al. 1991).

Fifty-one alleles previously reported to occur in North American stocks (Shaklee et al. 1991) were not observed in the eight collections from Russia analyzed here (Tables 2 and 3). Because most of these alleles are apparently rare in North America, this result probably also reflects the much smaller number of fish from Russia that were analyzed. In contrast, thirteen alleles found in the Russian samples had not been reported previously from North American odd-year pink salmon populations (Tables 2 and 3). While these alleles are rare in the Russian populations, this result could not be due to differences in sample size, and therefore, is probably indicative of real genetic differences between odd-year pink salmon populations from the two sides of the north Pacific Ocean.

The χ^2-tests revealing slight but significant heterogeneity in allelic composition among the eight collections of odd-year pink salmon from Russia indicate that the spawning populations in the different rivers are not part of a single, panmictic stock. This result is entirely consistent with the life history of pink salmon, the strong homing behavior they exhibit, and previous reports in the literature documenting genetic divergence among pink salmon stocks from different river systems (Altukov et al. 1983; Beacham et al. 1985a, 1988; Gharrett et al. 1988; Shaklee et al. 1991; Glubokovskii et al. 1990; Varnavskaya 1992; Varnavskaya and Beacham 1992). However, we could find no clear evidence of a geographic basis for the genetic heterogeneity observed among

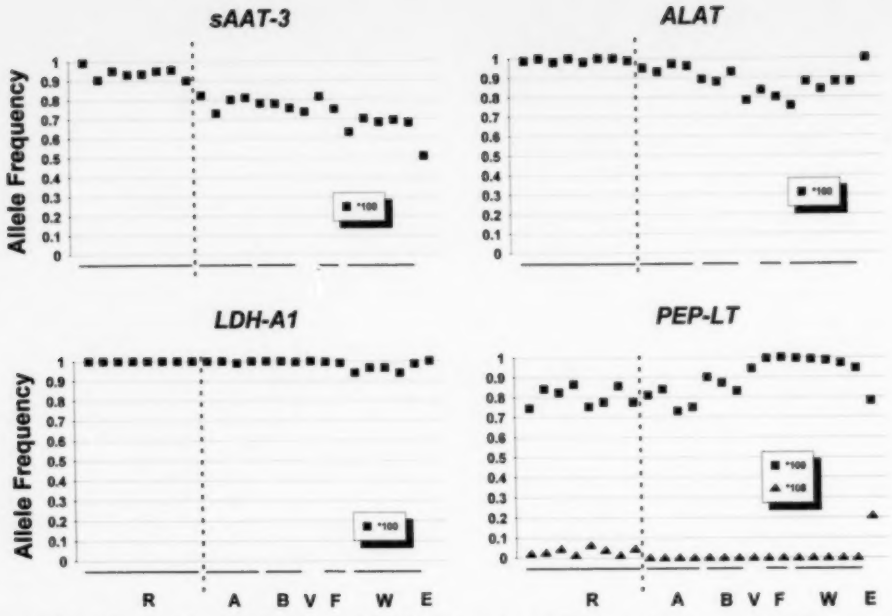

FIG. 3. Allele frequencies at four loci in eight Russian and 15 North American collections of odd-year pink salmon and one collection of even-year North American pink salmon. R = Russia; A = Alaska; B = northern and central British Columbia; V = Vancouver Island; F = Fraser River; W = Washington; E = Snohomish River even year. The order of collections (from left to right) in this figure is the same as the order in which they are listed in Table 1.

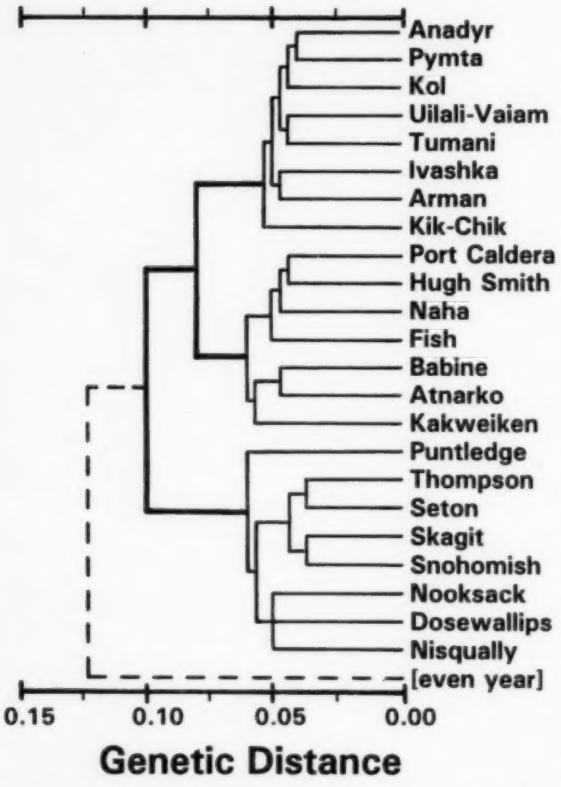

FIG. 4. UPGMA dendrogram of Cavalli-Sforza and Edwards (1967) chord distances using the 33 loci listed in Appendix 1. The three major branches of odd-year pink salmon (Russia, Alaska + northern Canada, and southern Canada + Washington) are indicated as bold lines while the branch leading to the single even-year stock included in this analysis is indicated as a broken line.

the populations from Russia. The absence of apparent geographic relationships among the populations could be a consequence of inaccurate estimates of allele frequencies owing to the relatively small sample sizes from each location (especially the Kik-Chik and Kol rivers). Alternatively, the major determinant of population structure of odd-year pink salmon in the northern part of Asia may not be isolation by distance, but rather one or more genetic, behavioral, or other environmental factor(s).

The Russian populations are, however, more similar to each other than to any of the populations from North America with which they were compared. Indeed, the dendrogram analysis (Fig. 4) of the larger data set (collections from Russia and from several regions of North America) exhibited a clear geographic pattern to the observed stock interrelationships. Varnavskaya and Beacham (1992) obtained a similar result when comparing Russian and North American odd-year populations of pink salmon using a limited data set consisting of only five polymorphic loci. Genetic similarity among geographically close populations is a reasonable expectation given a model of genetic isolation by distance (Wright 1978), and has been reported for stocks of chinook (Utter et al. 1989; Bartley and Gall 1990) and pink salmon (Shaklee et al. 1991). In contrast, adjacent stocks of sockeye salmon often exhibit very large genetic differences (Foote et al. 1989; G.A. Winans, P.B. Aebersold, and R.S. Waples, unpublished data).

The present analysis, based on 558 fish from the Kamchatka region, provides a preliminary view of the genetic

characteristics and population relationships of odd-year pink salmon from Russia. However, data for additional samples from Kamchatka, from other areas of Russia, and from many more Alaskan locations will be needed before a reliable and comprehensive understanding emerges.

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APPENDIX Allele frequencies at 33 variable gene loci in 15 collections of odd-year and one collection of even-year pink salmon from North America. (N) = number of fish successfully screened.

	Population															
Locus	Port Caldera	Fish Creek	Naha River	Hugh Smith	Babine R.	Kakweiken Atnarko		Thompson Puntledge		Seton	Nooksack Skagit		Snohomish Dosewallips		Nisqually Snohomish	even
mAAT-1																
*-100	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000
*-83	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(99)	(94)	(100)	(98)	(100)	(100)	(100)	(100)	(93)	(98)	(99)	(73)
mAAT-2																
*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.990	1.000	1.000	1.000	1.000	1.000	1.000
*-69	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(99)	(85)	(99)	(98)	(78)	(98)	(95)	(96)	(83)	(98)	(84)	(70)
sAAT-1,2																
*100	1.000	0.990	1.000	0.995	1.000	0.994	0.990	0.997	0.997	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*110	0.000	0.000	0.000	0.000	0.000	0.003	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*69	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*83	0.000	0.010	0.000	0.005	0.000	0.003	0.005	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(99)	(88)	(100)	(98)	(95)	(92)	(98)	(94)	(59)	(85)	(92)	(50)
sAAT-3																
*100	0.823	0.729	0.800	0.810	0.780	0.778	0.755	0.735	0.815	0.750	0.630	0.700	0.680	0.692	0.677	0.507
*91	0.177	0.271	0.200	0.190	0.220	0.222	0.245	0.265	0.185	0.250	0.370	0.300	0.320	0.308	0.323	0.493
(N)	(48)	(48)	(50)	(50)	(100)	(99)	(100)	(98)	(100)	(98)	(100)	(100)	(100)	(99)	(99)	(75)
sAAT-4																
*100	0.400	0.510	0.362	0.380	0.487	0.383	0.590	0.587	0.575	0.535	0.551	0.625	0.640	0.626	0.640	0.419
*190	0.590	0.490	0.638	0.610	0.513	0.617	0.410	0.413	0.425	0.465	0.449	0.375	0.360	0.374	0.360	0.581
*267	0.010	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(N)	(50)	(48)	(47)	(50)	(75)	(81)	(100)	(92)	(100)	(99)	(99)	(100)	(100)	(99)	(100)	(74)
ADA-2																
*100	0.900	0.860	0.870	0.870	0.965	0.939	0.920	0.925	0.900	0.910	0.940	0.860	0.899	0.980	0.945	0.980
*110	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.035	0.005	0.000	0.025	0.025	0.000	0.020	0.000
*90	0.100	0.140	0.130	0.130	0.035	0.056	0.080	0.075	0.065	0.085	0.060	0.115	0.076	0.020	0.035	0.020
(N)	(50)	(50)	(50)	(50)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(99)	(100)	(75)
sAH																
*100	1.000	1.000	1.000	1.000	1.000	1.000	0.985	0.905	0.975	0.955	0.985	0.970	0.990	0.980	0.964	1.000
*115	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.095	0.025	0.045	0.015	0.025	0.010	0.020	0.036	0.000
*90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(86)	(99)	(100)	(100)	(100)	(100)	(99)	(100)	(100)	(99)	(98)	(74)
ALAT																
*100	0.950	0.930	0.970	0.960	0.892	0.879	0.930	0.783	0.835	0.800	0.755	0.880	0.840	0.879	0.879	1.000
*111	0.010	0.030	0.010	0.010	0.015	0.010	0.005	0.000	0.005	0.005	0.010	0.010	0.035	0.000	0.005	0.000
*87	0.040	0.040	0.020	0.030	0.093	0.111	0.065	0.217	0.160	0.195	0.235	0.110	0.125	0.121	0.116	0.000
(N)	(50)	(50)	(50)	(50)	(97)	(99)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(99)	(99)	(75)

APPENDIX (cont.)

	Population															
Locus	Port Caldera	Fish Creek	Naha River	Hugh Smith	Babine R.	Atnarko	Kakweiken	Puntledge	Thompson	Seton	Nooksack	Skagit	Snohomish	Dosewallips	Nisqually	Snohomish even
CK-A1																
*100	1.000	1.000	1.000	1.000	1.000	0.985	1.000	1.000	0.995	0.985	0.980	0.985	0.995	0.995	1.000	1.000
*66	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.005	0.010	0.020	0.015	0.005	0.005	0.000	0.000
*74	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(49)	(50)	(93)	(99)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
FDHG																
*100	0.980	0.940	0.970	0.970	0.935	0.960	0.985	0.895	0.970	0.990	0.920	0.985	0.965	0.939	0.915	1.000
*138	0.020	0.060	0.030	0.030	0.065	0.040	0.015	0.105	0.030	0.010	0.080	0.015	0.020	0.056	0.085	0.000
*58	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.005	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
GDA																
*100	0.420	0.439	0.449	0.390		0.464	0.430	0.285	0.325	0.370	0.500	0.310	0.325	0.409	0.425	0.764
*108	0.450	0.429	0.408	0.470	no	0.351	0.370	0.450	0.460	0.405	0.309	0.490	0.470	0.465	0.445	0.236
*113	0.130	0.133	0.143	0.140	data[a]	0.186	0.200	0.265	0.215	0.225	0.191	0.195	0.205	0.126	0.130	0.000
*82	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
(N)	(50)	(49)	(49)	(50)		(97)	(100)	(100)	(100)	(100)	(97)	(100)	(100)	(99)	(100)	(74)
GPI-A																
*100	1.000	0.990	0.990	1.000	0.995	0.978	0.985	1.000	1.000	1.000	0.995	0.995	1.000	1.000	1.000	1.000
*108	0.000	0.010	0.000	0.000	0.005	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*91	0.000	0.000	0.010	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.005	0.005	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(95)	(92)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(98)	(100)	(74)
GPI-B1,2																
*100	0.995	1.000	0.984	1.000	1.000	0.994	1.000	0.990	0.983	0.977	0.995	0.987	0.990	0.959	0.992	1.000
*200	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.010	0.018	0.022	0.005	0.012	0.010	0.041	0.008	0.000
*-64	0.005	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*25	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(49)	(50)	(91)	(94)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
GR																
*100	1.000	1.000	1.000	0.990	1.000	0.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*109	0.000	0.000	0.000	0.010	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(100)	(98)	(94)	(100)	(100)	(99)	(99)	(90)	(92)	(92)	(77)	(74)
G3PDH-1																
*-100	0.890	0.830	0.900	0.810	0.970	0.889	0.880	0.835	0.965	0.955	0.935	0.950	0.935	0.864	0.970	0.847
*60	0.100	0.160	0.090	0.190	0.025	0.086	0.120	0.165	0.030	0.040	0.065	0.050	0.065	0.136	0.030	0.153
*20	0.010	0.010	0.010	0.000	0.000	0.025	0.015	0.000	0.005	0.005	0.000	0.000	0.000	0.000	0.000	0.000
*200	0.000	0.000	0.000	0.000	0.005	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(99)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
mIDHP-1																
*100	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000	1.000	1.000	0.995	1.000	1.000	1.000	1.000
*165	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(99)	(98)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(99)	(67)

APPENDIX (cont.)

Locus	Population															
	Port Caldera	Fish Creek	Naha River	Hugh Smith R.	Babine	Kakweiken Atnarko		Thompson Puntledge		Seton	Nooksack Skagit		Snohomish Dosewallips		Nisqually Snohomish even	
LDH-A1																
*-100	1.000	1.000	0.990	1.000	1.000	1.000	0.995	1.000	0.995	0.990	0.940	0.965	0.965	0.939	0.985	1.000
*-250	0.000	0.000	0.010	0.000	0.000	0.000	0.005	0.000	0.005	0.010	0.060	0.035	0.035	0.061	0.015	0.000
(N)	(50)	(50)	(49)	(50)	(97)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(74)
LDH-B1																
*100	1.000	1.000	0.990	1.000	1.000	1.000	1.000	1.000	0.990	1.000	1.000	0.985	1.000	1.000	1.000	1.000
*192	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.015	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
LDH-B2																
*100	1.000	1.000	1.000	1.000	1.000	0.995	0.975	0.995	0.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*132	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*74	0.000	0.000	0.000	0.000	0.000	0.005	0.015	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
LDH-C																
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
(N)	(50)	(50)	(50)	(50)	(100)	(99)	(100)	(98)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
sMDH-A1,2																
*100	1.000	0.995	1.000	1.000	0.997	1.000	0.997	0.997	0.997	0.992	1.000	0.995	0.997	0.997	1.000	1.000
*152	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
*-35	0.000	0.000	0.000	0.000	0.003	0.000	0.003	0.003	0.003	0.008	0.000	0.003	0.003	0.003	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(72)
sMDH-B1,2																
*100	0.940	0.970	0.965	0.935	0.968	0.950	0.942	0.995	0.995	0.990	0.985	0.990	0.975	0.967	0.992	0.983
*124	0.025	0.020	0.010	0.025	0.022	0.030	0.028	0.005	0.003	0.000	0.015	0.003	0.010	0.033	0.005	0.016
*70	0.035	0.010	0.025	0.040	0.010	0.020	0.030	0.000	0.003	0.010	0.000	0.008	0.015	0.000	0.003	0.000
(N)	(50)	(50)	(50)	(50)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
sMEP-1																
*100	0.990	0.970	0.980	0.980	0.970	0.985	0.985	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.875
*123	0.010	0.030	0.020	0.020	0.030	0.015	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125
(N)	(50)	(50)	(50)	(50)	(99)	(99)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(64)
sMEP-2																
*100	1.000	1.000	1.000	1.000	1.000	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*70	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(99)	(99)	(100)	(98)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
PEPB-1																
*100	0.980	0.970	0.990	1.000	1.000	1.000	0.984	1.000	1.000	1.000	0.990	1.000	0.995	1.000	0.995	0.986
*138	0.010	0.030	0.010	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.005	0.014
*50	0.010	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(47)	(90)	(94)	(95)	(100)	(97)	(99)	(98)	(100)	(97)	(96)	(70)

APPENDIX (cont.)

	Population															
Locus	Port Caldera	Fish Creek	Naha River	Hugh Smith R.	Babine	Kakweiken	Atnarko	Puntledge	Thompson	Seton	Nooksack	Skagit	Snohomish	Dosewallips	Nisqually	Snohomish even
PEPD-2																
*100	0.650	0.620	0.670	0.780	0.663	0.677	0.660	0.840	0.815	0.820	0.810	0.820	0.865	0.778	0.667	0.640
*120	0.200	0.240	0.130	0.090	0.245	0.192	0.165	0.075	0.130	0.145	0.145	0.115	0.090	0.187	0.283	0.100
*80	0.150	0.140	0.200	0.130	0.092	0.131	0.175	0.085	0.055	0.035	0.045	0.065	0.045	0.035	0.051	0.260
(N)	(50)	(50)	(50)	(50)	(98)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(99)	(75)
PEP-LT																
*100	0.810	0.840	0.730	0.750	0.900	0.872	0.830	0.944	0.995	1.000	0.995	0.990	0.985	0.970	0.945	0.780
*108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.207
*90	0.190	0.160	0.270	0.250	0.100	0.128	0.170	0.056	0.005	0.000	0.005	0.010	0.015	0.030	0.055	0.013
(N)	(50)	(50)	(50)	(50)	(30)	(98)	(100)	(99)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
PGDH																
*100	0.890	0.880	0.900	0.920	0.847	0.833	0.820	0.670	0.770	0.705	0.750	0.710	0.765	0.677	0.710	0.700
*94	0.090	0.090	0.060	0.070	0.153	0.167	0.180	0.330	0.230	0.295	0.250	0.290	0.235	0.323	0.290	0.247
*90	0.020	0.030	0.040	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053
(N)	(50)	(50)	(50)	(50)	(59)	(99)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(99)	(100)	(75)
PGM-2																
*100	0.910	0.950	0.960	0.910	0.985	0.939	0.895	0.805	0.820	0.825	0.884	0.830	0.795	0.828	0.930	1.000
*155	0.090	0.050	0.040	0.090	0.015	0.061	0.105	0.195	0.180	0.175	0.116	0.170	0.200	0.172	0.070	0.000
*25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
(N)	(50)	(50)	(50)	(50)	(100)	(99)	(100)	(100)	(100)	(100)	(99)	(100)	(100)	(99)	(100)	(75)

* = data for this locus from a 1989 collection from Babine (Shaklee et al. 1991) were substituted to allow the genetic distance and UPGMA dendrogram analyses.

Variation in Mitochondrial DNA and Allozymes Discriminates Early and Late Forms of Chinook Salmon (*Oncorhynchus tshawytscha*) in the Kenai and Kasilof Rivers, Alaska[1]

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Genetic differences between early and late forms of Alaskan chinook salmon (*Oncorhynchus tshawytscha*) were identified using two genetic approaches: mitochondrial DNA (mtDNA) analysis, and protein electrophoresis. Study populations consisted of early and late runs in each of the Kenai and Kasilof rivers in Alaska, and a population from the Minam River, Oregon. Two segments of mtDNA were amplified using the polymerase chain reaction (PCR) and digested with 14–16 restriction enzymes. Results showed that early runs were genetically similar to each other but different from the late runs. The late runs were different from each other based on the frequency of the common haplotypes. Frequency differences in shared haplotypes together with the presence of a unique haplotype separated the Minam River stock from those in Alaska. In the protein analysis, each population was examined at 30 allozyme loci. Based on 14 polymorphic loci, Minam River salmon were genetically distinct from the Alaskan populations. Within the Alaskan populations, early runs were most similar to each other but different from the late runs; the late runs were also genetically most similar to each other. Both mtDNA and allozyme analysis suggest that chinook salmon may segregate into genetically different early and late forms within a drainage.

Les auteurs ont identifié des différences génétiques chez des populations de saumon quinnat (*Oncorhynchus tshawytscha*) de l'Alaska à montaison hâtive et tardive en utilisant deux approches génétiques, soit l'analyse de l'ADN mitochondrial (ADNmt) et l'électrophorèse des protéines. Ces populations proviennent des rivières Kenai et Kasilof, en Alaska; une population de la rivière Minam, en Oregon, a aussi été étudiée. Deux segments d'ADNmt ont été amplifiés par réaction en chaîne de la polymérase (PCR), puis hydrolysés à l'aide de 14 à 16 enzymes de restriction. Les résultats ont révélé que les populations à montaison hâtive étaient génétiquement semblables, mais différentes des populations à montaison tardive. Ces dernières n'étaient pas semblables, du moins d'après la fréquence des haplotypes communs. Les différences dans la fréquence des haplotypes communs et la présence d'un haplotype unique différencient le stock de la rivière Minam des stocks de l'Alaska. Dans le cadre de l'analyse des protéines, 30 loci codeurs d'allozymes ont été examinés chez chaque population. D'après 14 loci polymorphes, les saumons de la rivière Minam sont distincts, au plan génétique, des populations de l'Alaska. Au sein des populations de l'Alaska, les populations à montaison hâtive se ressemblaient le plus, mais différaient des populations à montaison tardive; ces dernières se ressemblaient aussi le plus au plan génétique. Les résultats des analyses de l'ADNmt et des allozymes portent à croire que l'espèce peut se séparer en formes à montaison hâtive et tardive génétiquement différentes à l'intérieur d'un même bassin versant.

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[3]Cooperators include National Biological Survey, Oregon State University, and the Oregon Department of Fish and Wildlife.

The tendency of chinook salmon (*Oncorhynchus tshawytscha*) to return and spawn in their river of origin (Quinn 1982; McIsaac and Quinn 1988) results in separate breeding populations that may be biochemically, morphologically, and ecologically different. Because conserving genetic diversity among populations of fish is critical to species survival (Meffe 1986; Nelson and Soule 1987; Allendorf et al. 1987), a first step towards genetic conservation is to identify those groups that constitute separate breeding populations (Larkin 1981). Although it is often difficult to determine whether morphological and ecological differences among chinook salmon populations reflect separate breeding groups, the consequences of ignoring such differences may be a loss of genetic diversity for the whole species.

Analysis of genetic variation has proven to be a useful technique in identifying separate breeding populations. Genotypic data inferred through protein electrophoresis have been successful in discriminating among broad geographical groups of chinook salmon (Allendorf and Phelps 1981; Kristiansson and McIntyre 1976; Winans 1989; Utter et al. 1973, 1989; Gharret et al. 1987).

Analysis of mitochondrial DNA (mtDNA) is an alternative way of examining genetic diversity among groups. Detecting variation within species and organizing individuals into matriarchal phylogenies is facilitated by the maternal inheritance of mtDNA (Hutchison et al. 1974; Giles et al. 1980; Gyllensten et al. 1985), its individual homogeneity (Avise et al. 1979), and its apparently high rate of sequence evolution (Brown et al. 1979). The fast rate of evolution, which appears to be approximately four times faster than that of nuclear genes (Birky et al. 1983; Wilson et al. 1985), offers a magnified view of the divergence between closely related populations (Gyllensten and Wilson 1987) and aids tremendously in the analysis of interspecific relationships. MtDNA analysis in fish has been successful in detecting differences both among and within species (Thomas et al. 1986; Cronin et al. 1993). However, few studies have examined mtDNA variation of morphologically and ecologically differentiated chinook salmon.

Our objective was to determine if genetic differences existed among chinook salmon that exhibit different ecological spawning characteristics. Chinook salmon populations from the Kasilof and Kenai rivers, Alaska (Fig. 1), were chosen for two reasons. First, both rivers support early- and late-run salmon. Cold winter water temperatures and a short summer growing season in Alaska presumably cause the runs to be concentrated over a 3-mo period (Burger et al. 1985). In the Kasilof River, the early run enters the river during June and the late run returns from late July through early September (Faurot and Jones 1990). Similarly, Kenai River early-run chinook enter the river through June whereas the late-run returns during July and early August (Burger et al. 1985). Late-run fish in both rivers spawn in the mainstems downstream of large lakes whereas early-run fish spawn in tributaries that are not influenced by lakes (Burger et al. 1985; Faurot and Jones 1990).

Geographical proximity was a second factor in choosing the Alaska study populations. If genetic differences existed among geographically close yet ecologically different populations, detectable genetic differences might also exist among other chinook salmon populations in Alaska. In that case, a baseline could be developed to determine the genetic origins of the stocks harvested in various commercial and sport fisheries.

Methods

Collection of Samples

Skeletal muscle, liver, heart, eye, and caudal fin tissues were collected from chinook salmon during the summers of 1990, 1991, and 1992 from the Kenai and Kasilof rivers and the Minam River, Oregon (Fig. 1). Tissues were stored at −80°C until analyzed.

In the Kenai River, postspawning early-run chinook salmon were collected on the spawning grounds in a tributary (Fig. 1) by dipnetting. Late-run fish were collected with drift nets on the spawning grounds downstream of Skilak Lake (Fig. 1). Additional samples of early- and late-run salmon were collected from angled fish at Poacher's Cove each week from June 4 through July 27, 1991 (Fig. 1). Based on previous studies of seasonal entry into the river (Hammarstrom 1981) and geographical spawning distribution (Burger et al. 1985), fish collected before July 1 were considered the early-run form whereas fish collected after July 1 were considered to be late-run salmon.

During field sampling at Poacher's Cove and prior to obtaining tissues for genetic analysis, 12 morphological characteristics were measured on each fish to determine if morphological differences existed between the early and late runs of chinook salmon in the Kenai River. These morphological features are a collection of those used by other investigators (Riddell and Leggett 1981; Beacham 1984; Taylor and McPhail 1985; Hénault and Fortin 1989). Some of these characters have been successfully used to separate stocks within a basin (Riddell and Leggett 1981; Beacham 1984). Measurements were: (1) distance from mideye to fork of tail; (2) distance from snout to fork of tail; (3) weight; (4) girth (circumference of fish at anterior insertion of dorsal fin); (5) circumference of caudal peduncle; (6) length of base of dorsal fin; (7) length of longest dorsal fin ray; (8) length of base of anal fin; (9) length of longest anal fin ray; (10) length of longest pelvic fin ray; (11) length of longest pectoral fin ray; and (12) length of adipose fin from anterior end of base to tip.

In the Kasilof River, tissues from adult early-run chinook salmon and their progeny were obtained from fish at the Crooked Creek Hatchery (Fig. 1). The hatchery has propagated early-run chinook salmon since 1974 from native chinook salmon that spawned in Crooked Creek (R. Och, Alaska Department of Fish and Game, personal communication). Tissues were obtained from adult late-run chinook salmon collected with drift nets on the spawning grounds downstream of Tustumena Lake (Fig. 1). Because fertilized ova from Kasilof late-run salmon were incubated (Alaska Department of Fish and Game) for fry releases in other drainages, tissues were also obtained from hatchery-reared progeny of the late-run stock.

Tissues from juvenile chinook salmon from the Minam River, Oregon, were used to examine regional differences among chinook salmon populations and to provide a relative scale for interpreting differences among Alaskan populations. The Minam River is a tributary to the Snake River. The distant geographical isolation of Minam River chinook salmon suggested a high probability of genetic difference between this stock and Alaskan chinook salmon.

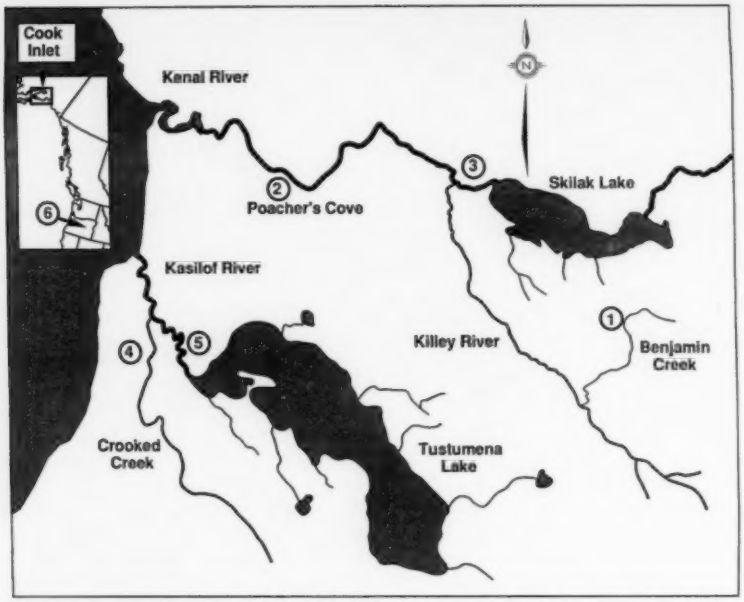

FIG. 1. Collection sites of chinook salmon samples in south-central Alaska and central Oregon, 1990 through 1992: (1) Kenai River early-run; (2) Kenai River early- and late-run; (3) Kenai River late-run; (4) Kasilof River early-run; (5) Kasilof River late-run; (6) Minam River (Insert, shows general sampling sites in Alaska and Oregon).

Tissue Analysis

Procedures described by Cronin et al. (1993) were used to extract, amplify, and digest mtDNA segments (NADH dehydrogenase subunit 1 (*ND-1*) and the control region) and to visualize restriction fragment patterns. Restriction enzymes used in the analysis of the *ND-1* and control region segments were *Ase*I, *Ava*II, *Bgl*I, *Bgl*II, *Bst*UI, *Dde*I, *Eco*RI, *Hae*II, *Hae*III, *Hin*cII, *Hin*dIII, *Msp*I, *Rsa*I, and *Xba*I. Additionally, *Bsa*JI and *Bst*NI were used only for the control region segment. The sizes of the restriction fragments were estimated by comparison with the standards (PhiX174 Am3cs70 virus DNA digested with *Hae*III or λ phage DNA digested with *Hin*dIII). Restriction fragment patterns produced by each of the mtDNA segment-restriction enzyme combinations were used to define composite haplotypes (Lansman et al. 1981).

Twenty fish from each of the Kasilof River chinook salmon runs were used to determine the repeatability of the mtDNA analysis techniques applied in this study. DNA was extracted and analyzed from muscle at the Oregon Cooperative Fisheries Research Unit laboratory, Oregon State University. Liver samples from the same fish were analyzed at the Alaska Fish and Wildlife Research Center laboratory in Anchorage, Alaska. Different tissues (muscle and liver) were used to determine if identical results could be achieved regardless of tissue type. The haplotype of each individual was identified at both labs and the results were examined for inconsistencies. Procedures for detecting genetic variations at 30 allozyme loci (Table 1) followed those of Aebersold et al. (1987).

Data Analysis

Based on seasonal entry into the rivers (Hammarstrom 1981; Faurot and Jones 1990) and geographical spawning distribution (Burger et al. 1985), data were partitioned into five groups: Kenai River early run, Kenai River late run, Kasilof River early run, Kasilof River late run, and Minam River. Tests of homogeneity using the likelihood of ratio statistic (G; Sokal and Rohlf 1981) were used to determine if haplotype frequency data as well as allele frequency data could be pooled for different years and maturity classes.

Among-Population Differences

Relationships among groups were defined by hierarchial tests of homogeneity, using the log likelihood ratio statistic (G; Nei 1973, 1975; Sokal and Rohlf 1981), for both haplotype and allele frequencies. The level of significance for each comparison within the hierarchy was calculated following the procedures of Sokal and Rohlf (1981).

Morphological differences between early- and late-run chinook salmon in the Kenai River were examined by analysis of variance (ANOVA). A random sample of 20 fish from each population was used in the analysis. A principal component analysis (PCA) was conducted to examine the effect overall fish size had on the 12 morphological characteristics measured. Removal of the first component of the PCA, which represents fish size, allowed us to determine if there was a difference in body shape between the early and the late runs of chinook salmon in the Kenai River.

Estimates of Within-Population Diversity

Genetic diversity within each population was estimated using both mtDNA and allozyme data. MtDNA haplotype and nucleotide diversity was estimated according to Nei (1987) and Nei and Tajima (1981) using haplotype frequencies. Within-population diversity based on allozyme data was estimated from mean heterozygosities and the percentage of polymorphic loci. Goodness-of-fit tests using Pearson's X^2 were used to determine departure from Hardy–Weinberg equilibrium.

TABLE 1. International Union of Biochemistry (I.U.B.) enzyme names (1984), Enzyme Commission (E.C.) numbers, loci, tissues, and buffers used in this study. Tissues: M = muscle; L = liver; E = eye; H = heart. Buffers: TBE = Tris–borate–EDTA-gel and tray buffer, pH 8.5; CAME = citric acid – EDTA gel and tray buffer, pH 6.8; CAMEN = citric acid – EDTA – NAD^+ gel and tray buffer, pH 6.8; TC-4 = Tris – citric acid gel and tray buffer, pH 5.8; KG = Tris–glycine gel and tray buffer, pH 8.4 (Wilmot et al. 1992).

I.U.B. enzyme name	E.C. No.	Locus	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	*mAAT-1**	H	CAMEN 6.8
		*sAAT-1,2**	H, M	CAME 6.8
Adenosine deaminase	3.5.4.4	*ADA-1**	H, M	KG, TC-4
		*ADA-2**	H, M	KG, TC-4
Aconitate hydratase	4.2.1.3	*sAH-1**	L	CAME 6.8, TC-4
Alanine aminotransferase	2.6.1.2	*ALAT**	H, M	KG
Creatine kinase	2.7.3.2	*CK-B**	M, E	KG
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B1**	M	KG, TBE
		*GPI-B2**	M	KG, TBE
		*GPI-A**	M	KG, TBE
Glutathione reductase	1.6.4.2	*GR**	H, M	TC-4
			E	CAME 6.8
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	*sIDHP-1**	H, M, L	TC-4
			M, E, L	CAME 6.8
		*sIDHP-2**	H, M, L	TC-4
			M, E, L	CAME 6.8
L-lactate dehydrogenase	1.1.1.27	*LDH-B1**	E, L	KG, CAME 6.8
		*LDH-B2**	E, L	KG, CAME 6.8
		*LDH-C**	E	KG
Malate dehydrogenase	1.1.1.37	*mMDH-1**	H	CAMEN 6.8
		*sMDH-A1,2**	H, M, E, L	CAME 6.8
		*sMDH-B1,2**	H, M, E, L	CAME 6.8
Malic enzyme ($NADP^+$)	1.1.1.40	*sMEP-1**	H, M	CAME 6.8
			L	TC-4
		*sMEP-2**	H, M	CAME 6.8
			L	TC-4
Dipeptidase	3.4.-.-	*PEPA**	E	KG, CAME 6.8
Tripeptide aminopeptidase	3.4.-.-	*PEPB-1**	H, M	KG, TC-4
Proline dipeptidase	3.4.13.9	*PEPD2**	H	CAME 6.8
			M, L	TC-4
Phosphoglucomutase	5.4.2.2	*PGM-1**	H, M	KG
			L	TC-4
		*PGM-2**	H, M	KG
			L	TC-4
Superoxide dismutase	1.15.1.1	*sSOD-1**	L	TC-4
			H, M	KG
Triose-phosphate isomerase	5.3.1.1	*TPI-1**	H, M, E	KG
		*TPI-2**	H, M, E	KG
		*TPI-3**	H, M, E	KG

Estimates of Between-Population Diversity

Genetic diversity between populations was also estimated using both mtDNA and allozyme data. MtDNA nucleotide divergence was estimated according to Nei (1987). Nei's genetic identity values (Nei 1972; 1978) were used to estimate allozyme diversity between populations. Phenograms based on both haplotype and allele frequencies were examined for mtDNA and allozyme similarities among populations. Phenograms were constructed from matrices of genetic identity values (Nei 1972, 1978) using the unweighted pair-group method with arithmetic averages (UPGMA) algorithm (Sneath and Sokal 1973). Cluster analysis of the mtDNA data was also conducted using nucleotide divergence values and compared with the phenogram based on haplotype frequencies.

Results

Results of the replicate testing were 100% repeatable between the two labs. The haplotype of each individual fish was consistently identified at both labs regardless of the type of tissue (liver or muscle) used in the analysis.

Within each population, both haplotype and allele frequency data from different years and maturity classes showed no statistically significant differences, allowing us to pool the data for each population. The mtDNA analysis revealed variable fragment patterns when the *ND-1* segment was digested with *Dde*I and *Rsa*I and the control region segment was digested with *Rsa*I (Table 2). The four different composite haplotypes (D1, D2, D3, D4) are described in Table 3 and their distribution among populations is shown in

TABLE 2. MtDNA segments with restriction site polymorphisms and the restriction fragment lengths. Fragment length is presented as number of base pairs.

MtDNA segment	Restriction site	Fragment length	Pattern	
Control region	*Rsa* I	1153	—	B
		887	A	—
		441	A	—
		252	—	B
ND-1	*Dde* I	475	A	—
		422	—	B
		349	A	B
		279	A	B
		258	A	B
		193	A	B
		183	—	B
	Rsa I	663	A	C
		542	—	C
		381	A	—
		361	A	C
		327	A	C
		305	A	—

TABLE 3. Composite haplotype definitions for chinook salmon. Letters refer to the banding patterns seen in individual samples. The banding patterns are shown in Table 2.

MtDNA segment	Restriction enzyme	Haplotype definition			
		D1	D2	D3	D4
Control region	*Rsa*I	A	A	A	B
ND-1	*Dde*I	A	A	B	A
	*Rsa*I	A	C	C	C

Table 4. Of the 30 allozyme loci examined, 16 were monomorphic for all populations. Of the remaining 14, five loci had low levels of polymorphism (frequency of the common allele >0.95); these were *AH-1**, *ALAT**, *LDH-B2**, *PEPD2**, and *PEPB-1**. Loci which displayed higher levels of polymorphism (frequency of the most common allele <0.95 in at least one population) were *ADA-1**, *sIDHP-1**, *sIDHP-2**, *sMDH-B1,2**, *sMEP-1**, *sMEP-2**, *PEPA**, *sSOD-1**, and *TPI-4** (Table 5). No deviations from Hardy–Weinberg equilibrium were observed ($P > 0.05$).

Among-Population Differences

Tests of homogeneity identified similar patterns of genetic differentiation for both mtDNA and allozyme data (Table 6). The mtDNA data indicated that the early runs in the Kenai and Kasilof rivers were genetically similar to each other but different from either of the late runs; the late runs were different from each other based on the frequency of the common haplotypes (haplotypes D1 and D2). Although all populations shared the D1 and D2 mtDNA haplotypes, the frequency difference of the shared haplotypes together with a unique haplotype (D3) in the Minam River stock allowed us to separate the Oregon population from those in Alaska. Because the Minam River population shared haplotypes D1 and D2 with the Alaska populations, it was genetically most similar to the two early runs in this respect. There was also a unique haplotype (D4) found at a low frequency in the Kenai River early-run population.

The allozyme data revealed genetic differences among the populations that were very similar to those determined by mtDNA analysis (Table 6). Among the Alaska populations, the test of homogeneity indicated that the two early runs were genetically most similar to each other but different from both of the late runs. The two late runs were statistically different from each other and the Minam River chinook were statistically different from the Alaska populations.

There was a significant difference in size of the fish from the Kenai River early and late runs. Based on all 12 morphological characteristics, late-run fish were larger than early-run salmon (Hotelling-T; $p < 0.0001$) (Table 7). The first component of the PCA accounted for 85.7% of the variation between groups while the second and third components accounted for 4.5% and 2.7% respectively. After accounting for the effect that fish size had on body shape by removing the first component, there was no statistically significant difference between early- and late-run salmon in the Kenai River. Consequently, we were unable to identify any differences in shape despite the clear difference in overall fish size.

Estimates of Within-Population Diversity

The most genetically diverse population was the Kenai River late run. Haplotype diversity within this population was 0.4806 and nucleotide diversity was 0.0044 (Table 4). Mean heterozygosity and the percentage of polymorphic loci were 0.037 and 40% (Table 5). Mean heterozygosity (0.034) and percentage of polymorphic loci (40%) in the Kenai early run were comparable but the early run had the lowest haplotype diversity (0.1716) as well as the lowest nucleotide diversity (0.0019) of all populations.

Estimates of Between-Population Diversity

Nucleotide divergence ranged from 0.0001 to 0.0096 and averaged 0.0021 (Table 4). Although these values are low, intraspecific mtDNA divergence values less than 0.01 have been reported for chinook salmon from Alaska and British Columbia (Wilson et al. 1987). The greatest divergence among populations occurred between the Kasilof River late run and the other four populations. The divergence of the Kasilof River late run was, on average, 2.5 times greater than the divergence among the other populations.

The divergence of the Kasilof River late run is clearly depicted in the mtDNA phenogram shown in Fig. 2. However, the phenogram depicting the allozyme similarities among the populations does not show a clear divergence of the Kasilof River late run. Instead, the populations appeared to cluster in accordance with their ecological spawning characteristics and geographic locations. The two early runs grouped separately from the two late runs, and a clear distinction exists between the Minam River and Alaska chinook salmon populations. The same groupings did not occur when cluster analysis was conducted using mtDNA data. In this case, the Minam River population was similar to the two Alaska early runs and the two late runs were different from each other. An identical mtDNA phenogram resulted when cluster analysis was conducted using the nucleotide divergence values.

TABLE 4. (A) Distribution of chinook salmon mtDNA haplotypes among locations in the Kenai, Kasilof, and Minam rivers and haplotype and nucleotide diversity within each population. Numbers of locations correspond to those in Fig. 1. A = adult; J = juvenile.

Sampling location	Sample group	Year sampled	Maturity	Composite haplotype				Haplotype diversity	Nucleotide diversity
				D1	D2	D3	D4		
1,2	Kenai early-run	1991	A	4	70	—	3	0.1716	0.0019
2,3	Kenai late-run	1990, 1991	A	52	76	—	—	0.4806	0.0044
4	Kasilof early-run	1990, 1991	A	4	21	—	—	0.3094	0.0030
		1991, 1992	J	4	29	—	—		
5	Kasilof late-run	1990, 1991, 1992	A	66	16	—	—	0.2809	0.0026
		1991, 1992	J	26	9	—	—		
6	Minam River	1990	J	1	21	3	—	0.2900	0.0028

(B) Nucleotide diversity (above diagonal) and divergence (below diagonal) among populations

	Kenai early-run	Kenai late-run	Kasilof early-run	Kasilof late-run	Minam River
Kenai early-run	—	0.0042	0.0025	0.0079	0.0024
Kenai later-run	0.0010	—	0.0043	0.0052	0.0048
Kasilof early-run	0.0001	0.0006	—	0.0073	0.0029
Kasilof late-run	0.0056	0.0018	0.0046	—	0.0085
Minam River	0.0001	0.0012	0.0001	0.0059	—

Discussion

Based on both mtDNA and allozyme analysis, we identified genetic differences among ecologically different forms of chinook salmon in the Kenai and Kasilof rivers. The largest genetic difference, based on the test of homogeneity using mtDNA data, occurred between the Kasilof River early and late runs. Genetic differences among salmon within a drainage have previously been reported based on allozyme analysis (Currens et al. 1990; Wilmot et al. 1992). However, we could not locate published accounts of mtDNA differences between populations in a drainage that are as temporally or spatially close as the early and late runs in the Kasilof River. The spawning times of early- and late-run chinook salmon in the Kasilof River differ by only about 6 weeks and their spawning grounds are separated by only 19 river kilometers.

Because the Kasilof River early run has been propagated in a hatchery since 1974, a founder effect as well as genetic drift could account for the differences we identified in the Kasilof River populations. To evaluate the potential for founder effect in hatchery populations (Waples 1990), the number of fish used as broodstock since 1974 in the Crooked Creek hatchery was examined. Twelve females (estimated fecundity = 8000) and 19 males from the naturally spawning population in Crooked Creek were used to start the hatchery population in 1974 (R. Och, Alaska Department of Fish and Game, personal communication). Chinook salmon take 2–5 yr to reach sexual maturity. As a result, adults from the naturally spawning population were used in the years following 1974 because the progeny of the initial hatchery population would not have returned to spawn for at least 2–5 yr. In 1991 and 1992, an average of 68.4 females (estimated fecundity = 6700) and 36.2 males of the fish returning to the hatchery were used to propagate the early run. Although the effective population size (N_e; Hartl and Clark 1989) for the initial spawning was relatively low (N_e = 29.42), continued use of wild spawning fish in the years immediately following 1974 and the presence of their haplotypes (D1 and D2) in similar frequencies in the Kenai River populations suggest that the hatchery influence does not account for all of the variability identified in the Kasilof River populations. The genetic difference between the early and late runs in the Kasilof River is further supported by the test of homogeneity (Table 6) and cluster analysis (Fig. 2) based on the allozyme data.

Genetic differences were also identified between ecologically different forms in the Kenai River. Both the test of homogeneity and the cluster analysis revealed genetic differences between the early and late runs.

Among the populations that exhibit ecologically similar spawning characteristics, tests of homogeneity between the two early runs in the Kenai and Kasilof rivers showed no statistically significant difference. Conversely, there was a statistically significant difference between the two late runs. The sampling design for obtaining fish from the Kenai River late-run population could explain the difference between the two late runs. Because all but 7 of the 128 fish categorized as late-run salmon were obtained in the lower section of the Kenai River at Poacher's Cove (Fig. 1), it is likely that we unintentionally included some early-run fish in the late-run category and artificially altered the haplotype frequencies within the Kenai River late-run group. Studies in previous years showed all fish collected at Poacher's Cove after July 1 to be late-run salmon (Burger et al. 1985; Hammarstrom 1981). During 1991 and 1992, however, early-run salmon may have migrated through the lower river to spawning tributaries after July 1. The only difference between the two late runs is the frequency of the shared haplotypes, D1 and D2. The D2 haplotype predominates in the early runs from both the Alaska rivers. The D1 haplotype is characteristic of late-run fish in both rivers. If Kenai River early-run fish

TABLE 5. Allelic frequencies at 14 polymorphic loci for chinnok salmon sampled from the Kenai, Kasilof, and Minam rivers from 1990 to 1992. The most populations. Allele mobility numbers separated with a slash

	ADA-1		*sAH-1*		*ALAT*		*sIDHP-1*			*sIDHP-2*			*LDH-B2*		*sMDH-B1, 2*	
Population	100	83	100	86	100	94	100	74	136	100	127	50	100	56	100	121/126
Kenai early-run	0.965	0.035	0.990	0.010	0.995	0.005	0.995	0.000	0.005	0.995	0.000	0.005	1.000	0.000	0.985	0.015
Kenai late-run	0.980	0.020	0.980	0.020	0.990	0.010	1.000	0.000	0.000	0.990	0.000	0.010	1.000	0.000	0.990	0.010
Kasilof early-run	0.940	0.060	1.000	0.000	0.985	0.015	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
Kasilof late-run	0.995	0.005	0.995	0.005	0.995	0.005	1.000	0.000	0.000	0.945	0.000	0.055	1.000	0.000	0.990	0.010
Minam River	0.970	0.030	1.000	0.000	1.000	0.000	0.900	0.100	0.000	0.965	0.030	0.005	0.990	0.010	0.895	0.105

(haplotype D2) were inadvertently included in the late-run group, the results would be an over abundance of D2 haplotypes in the Kenai River late-run group. This could account for the haplotype frequency difference identified between the two late runs.

The sampling design in the Kenai River might also account for the relatively high values of within-population diversity in the Kenai River late-run. The likely inclusion of some early-run fish in the late-run category may have artificially increased estimates of genetic variability of Kenai River late-run salmon.

Although both mtDNA and allozyme analysis identified similar genetic differences among the populations, genetic identity values indicate that mtDNA analysis was more powerful in discriminating between the groups on a locus by locus basis. Nei's unbiased genetic identity, based on haplotype data (1978) ranged from 0.454 to 0.998 in the mtDNA phenogram. Separation among populations according to allozyme data occurred between 0.994 and 0.999 (Fig. 2). Put into perspective, all the groupings in the allozyme phenogram occur in the same amount of space as that between the Minam River and Kenai River early-run populations in the mtDNA phenogram.

Although the magnitude of the differences was greater using the mtDNA data, the two phenograms were different. The difference may be a consequence of the reduced effective population size of mtDNA (one quarter that of nuclear DNA) and the resulting increased susceptibility to genetic drift and bottleneck effects. The difference may also be attributed to the number of loci used in constructing the phenograms. The phenogram depicting the allozyme differences uses 14 loci whereas the mtDNA phenogram is based on only one locus. Based on the single mtDNA locus, the degree of separation between the populations was greater than any one, or all, of the 14 polymorphic allozyme loci. The *MEP-2** locus had the greatest frequency difference between populations (Table 5). Nevertheless, when cluster analysis was conducted using this locus no genetic difference was identified among the Alaska populations (Nei's Identity = 1). A distinction was made between the Alaska populations as a whole and Minam River chinook salmon (Nei's Identity = 0.849), however the magnitude of the separation was still less than those identified using mtDNA analysis.

Ecology

Natural selection for differences in spawning habitat may explain the genetic differences between the early and late runs in the Kenai and Kasilof rivers. Late-run salmon return to spawn in the mainstem of their respective rivers downstream of glacier-fed lakes. The thermal capacity of lakes may maintain elevated temperatures downstream (Carmack et al. 1979) to enable successful spawning late in the year. Late-run chinook salmon in the Kenai and Kasilof rivers are the latest known spawning populations in south-central Alaska. Spawning by late-run chinook salmon peaks in the Kenai River during late August (Burger et al. 1985) and through mid-September in the Kasilof River (C. Burger, unpublished data). The warmer temperatures maintained by the lakes may limit spawning late in the year to areas downstream of lakes (Burger et al. 1985). Thus, late-run fish could not successfully spawn in the same upstream areas as early runs due to suboptimal (colder) water temperatures late in the year.

The importance of temperature in governing the spawning activity of rainbow trout (*Oncorhynchus mykiss*) was demonstrated by Morrison and Smith (1986). They successfully altered the spawning time by manipulating water temperatures. Temperature affects the development rate and viability of gametes prior to spawning and also influences the rate of embryonic development and subsequent emergence of the fry during optimal environmental conditions. Water temperature may be a factor in the reproductive isolation identified between early- and late-run chinook salmon in the Kenai and Kasilof rivers. A similar conclusion was reached in explaining the occurrence of a genetically unique late run of sockeye salmon in the upper Kasilof River (C. Burger, unpublished data).

Natural selection for differences in spawning habitat may explain the genetic isolation between the populations of chinook salmon in the Kenai and Kasilof rivers. It may also account for the difference in body size observed between the early and late runs in the Kenai River. Late-run chinook salmon spawn almost exclusively in the mainstem of the Kenai River (as opposed to the tributaries used by the early run) (Burger et al. 1985), and natural selection may have favored a larger body size. We theorize that larger body size may be an adaptation to the greater water velocities found in the mainstem of the Kenai River. This idea is supported by studies on chum salmon (*O. keta*) (Beacham 1984), brown trout (*Salmo trutta*) (Yevsin 1977), and Atlantic salmon (*S. salar*) (Jones 1975; Schaffer and Elson 1975; Riddell and Leggett 1981). Breeding experiments conducted under controlled conditions have shown that these interpopulational differences in morphological characteristics are heritable and represent adaptations to natal rearing environments (Riddell and Leggett 1981). Further information is needed to verify that the same adaptations have occurred in the Kenai River populations. Nevertheless, difference in fish size between the two runs correlates with the genetic

common allele is designated as 100, and other alleles assigned numbers according to their mobility relative to the 100 allele. $N = 100$ for all five indicate that the data for those two alleles have been pooled.

sMEP-1		*sMEP-2*		*PEPA*		*PEPD2*		*PEPB-1*		*sSOD-1*		*TPI-4*		Summary	
100	92/86	100	78	100	90	100	83	100	130	100	−260	100	104	Mean hetero-zygosity	% of polymorphic loci
0.090	0.910	0.615	0.385	0.990	0.010	1.000	0.000	0.975	0.025	0.990	0.010	0.930	0.070	0.034	40.0
0.045	0.995	0.615	0.385	0.910	0.090	0.995	0.005	0.990	0.010	0.900	0.100	0.960	0.040	0.037	40.0
0.105	0.895	0.590	0.410	0.995	0.005	0.980	0.020	0.980	0.020	0.990	0.010	0.855	0.145	0.040	30.0
0.025	0.975	0.640	0.360	0.980	0.020	1.000	0.000	0.980	0.020	0.940	0.060	1.000	0.000	0.029	33.3
0.060	0.940	1.000	0.000	0.990	0.010	1.000	0.000	0.990	0.010	0.880	0.120	0.915	0.085	0.036	33.3

TABLE 6. Results of hierarchial tests of homogeneity using mtDNA (haplotypes D1, D2, D3, D4) and allozyme (14 polymorphic loci) data among and within the chinook salmon populations in the Kenai, Kasilof, and Minam rivers, and tests of homogeneity between the two early and two late runs in the Kenai and Kasilof rivers. G = log-likelihood ratio statistic, df = degrees of freedom, p = probability of rejecting the null hypothesis when it is true based on the observed G and df, LOS = level of significance for each test in the hierarchy where the overall LOS = 0.05)

	Mitochondrial DNA				Allozyme			
Goup	G	df	p	LOS	G	df	p	LOS
Among basins/runs	181.85	12	<0.001	0.050	358.12	32	<0.05	0.050
Within basins								
Kenai River	40.40	2	<0.001	0.013	44.90	16	<0.05	0.013
Kasilof River	71.04	1	<0.001	0.013	102.60	16	<0.05	0.013
Between like runs								
Early runs	6.18	2	0.045	0.013	24.73	16	>0.05	0.013
Late runs	37.71	1	<0.001	0.013	38.47	16	<0.05	0.013

TABLE 7. Means (with SE given in parentheses) for the 12 morphological characteristics of the Kenai River early- and late-run chinook salmon. All measurements are in centimeters except for weight, which is in kilograms. $N = 20$ for each population.

Morphological character	Early-run	Late-run
Distance from mideye to fork of tail	88.61 (1.92)	99.15 (1.16)
Distance from snout to fork of tail	97.28 (2.25)	110.92 (1.48)
Weight	10.56 (0.63)	15.61 (0.57)
Girth	57.65 (1.23)	67.21 (0.89)
Circumference of caudal peduncle	20.47 (0.46)	22.89 (1.36)
Length of base of dorsal fin	10.59 (0.26)	12.33 (0.26)
Length of longest dorsal fin ray	11.08 (0.29)	13.02 (1.02)
Length of base of anal fin	11.85 (0.26)	13.51 (0.27)
Length of longest anal fin ray	8.60 (0.25)	10.36 (0.20)
Length of longest pelvic fin ray	9.78 (0.23)	11.69 (0.18)
Length of longest pectoral fin ray	11.76 (0.26)	13.44 (0.22)
Length of adipose fin from anterior end of base to tip	5.76 (0.28)	6.58 (0.32)

differences we identified between populations that exhibit different ecological spawning characteristics.

Colonization and Evolution

Our results can be used to support either of two current theories concerning the colonization and evolution of the Alaska chinook salmon. One theory (Bartley and Gall 1990) suggests that colonization of glaciated areas in Alaska took place approximately 10 000 yr ago by chinook salmon from the Columbia River and Bering Sea. The similarity in distribution of the D1 and D2 haplotypes among Minam River and the two early runs in the Kenai and Kasilof rivers supports this hypothesis. The frequency of the shared haplotypes suggests that an ancestral form from the Columbia River may have colonized the early runs in the Kenai and Kasilof rivers. Genetically different late runs in both rivers may have diverged later due to local adaptation and reproductive isolation. Evidence in favor of the divergence of the two late runs is shown by the cluster analysis based on allozyme data. The two early and two late runs were more similar to each other. (Fig. 2).

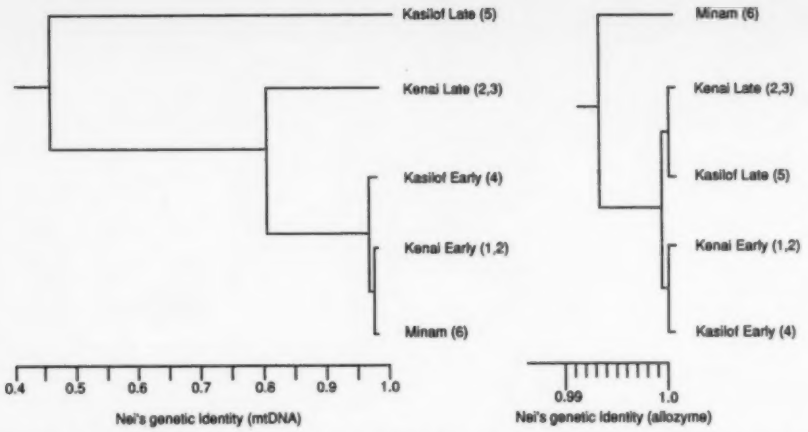

FIG. 2. Phenograms showing genetic relationships among five populations of chinook salmon from the Kenai, Kasilof, and Minam rivers based on both mtDNA haplotype and allozyme frequency data. Numbers in parentheses correspond to locations shown in Fig. 1.

It is also possible that recolonization occurred out of a central Alaskan refugium (Cronin et al. 1993; Gharrett et al. 1987). If populations with similar mtDNA lineages survived in each of the Alaskan and Columbia River refugia, then the shared haplotypes in the Minam and Alaskan populations could be explained. As previously mentioned, a more recent divergence of the two late runs in the Kenai and Kasilof rivers would explain the within-basin differences we identified.

Equally plausible is that allopatric divergence may account for the genetic differences we identified. The potential existence of an Alaskan and Columbian refugia during glaciation may have resulted in genetic differences due to reproductive isolation, with the Alaskan fish utilizing lakes to facilitate successful spawning late in the year and Columbian fish spawning in the rivers early in the year. The Columbian fish could have subsequently spread north with the retreat of the glaciers and either displaced any river spawning populations in Alaska or simply colonized unused river spawning habitat.

Summary

Conserving genetic diversity among populations of fish is critical to species survival (Nelson and Soule 1987; Meffe 1986; Allendorf et al. 1987). The first step towards genetic conservation is to identify those groups that constitute separate breeding populations (Larkin 1981). Our results demonstrate the usefulness of mtDNA and protein electrophoresis techniques in identifying separate breeding populations that are temporally and spatially close. Other studies have used mtDNA techhniques to identify differences among stocks of the same species, yet little published data exist that identify significant genetic differences between runs within the same drainage. Furthermore, our ability to consistently identify haplotypes of each individual at the two laboratories demonstrates the repeatability of the mtDNA techniques we used. The replicate tests also demonstrated that mtDNA from muscle or liver tissue can be used to achieve identical results.

Results of this study suggest that sufficient variation exists to develop a genetic baseline for stocks of chinook salmon originating in Cook Inlet in Alaska. This information would aid in the management of the species in Alaska and contribute to the coast-wide management of the species. Our results also demonstrate the potential loss of genetic diversity that could occur by ignoring ecological and morphological differences that exist among chinook salmon within a drainage or among drainages.

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Genetic Relationships Among *Salvelinus* Species Inferred from Allozyme Data

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We studied phylogenetic relationships in the genus *Salvelinus* with emphasis on *S. alpinus*, *S. malma*, and *S. confluentus*. Sixty allozyme loci were resolved in 422 individuals representing six species from a worldwide distribution. We used phenetic (UPGMA), phylogenetic (Fitch and Margoliash), and maximum-likelihood techniques to derive estimates of *Salvelinus* phylogeny. Three almost identical topologies were produced, but we chose the Fitch and Margoliash topology as the best phylogenetic estimate. The distances used to construct this tree assume that gene frequencies are affected only by genetic drift, which we argued was the dominant speciation force in *Salvelinus*. This topology was also the shortest. Jackknifing the Fitch and Margoliash tree resolved the branching order among the three *Salvelinus* subgenera: *S.* [*Baione*], *S.* [*Cristivomer*], and *S.* [*Salvelinus*]. It also revealed two sister groups within the *Salvelinus* subgenus: *S. leucomaenis* – *S. confluentus* and *S. alpinus* – *S. malma*.

Les auteurs ont étudié les relations phylogénétiques chez les espèces du genre *Salvelinus*, en particulier *S. alpinus*, *S. malma* et *S. confluentus*. Ils ont identifié 60 loci codeurs d'allozymes chez 422 poissons de six espèces représentant la répartition mondiale du genre, et se sont servis d'analyses phénétiques (UPGMA), phylogénétiques (Fitch et Margoliash) et du maximum de vraisemblance pour obtenir des estimations de la phylogénie de *Salvelinus*. Ayant obtenu des topologies presque identiques, ils ont choisi la topologie de Fitch et et Margoliash comme meilleure estimation phylogénétique. Les distances utilisées pour construire cet arbre supposent que seule la dérive génique a une incidence sur la fréquence allélique; les auteurs argumentent que la dérive génique est la force de spéciation dominante chez *Salvelinus*. Cette topologie était aussi la moins élaborée. L'application de la technique du canif à l'arbre de Fitch et et Margoliash a permis d'établir l'ordre de ramification des trois sous-genres de *Salvelinus* : *S.* [*Baione*], *S.* [*Cristivomer*] et *S.* [*Salvelinus*]. Cette analyse a aussi révélé deux groupes frères au sein du sous-genre *Salvelinus* : *S. leucomaenis* – *S. confluentus* et *S. alpinus* – *S. malma*.

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The evolutionary relationships of charrs (the salmonid genus *Salvelinus*) have not been adequately resolved, particularly among the three subgenera and among species within one subgenus, *S.* [*Salvelinus*]. This is because of the morphological and ecological plasticity that the genus exhibits throughout its range. Several morphotypes of charrs, each with its own niche, can coexist within one watershed (Morrow 1980; Power 1980; Magnusson and Ferguson 1987; Parker and Johnson 1991). Many interesting questions can be asked about mechanisms of adaptation and speciation using charrs as a model; however, this type of research has been limited because of disagreement about the number and relationships of recognized taxa.

Salvelinus contains three subgenera: lake trout (*S. namaycush*) in the subgenus *S.* [*Cristivomer*] (Behnke 1972); brook trout (*S. fontinalis*) in the subgenus *S.* [*Baione*] (Vladykov 1954, 1963); and all other charrs, including arctic charr (*S. alpinus*), Dolly Varden (*S. malma*), bull trout (*S. confluentus*), and white-spotted r (*S. leucomaenis*), in the subgenus *S.* [*Salvelinus*] (Vladykov 1954, 1963; Behnke 1972, 1980, 1984).

Savvaitova (1980) hypothesized that *S. namaycush*, *S. fontinalis*, and *S. leucomaenis* shared a common ancestor distributed on the Atlantic and Pacific coasts of North America. Geographic isolation resulted in speciation of these three taxa. *Salvelinus namaycush* and *S. fontinalis* diverged in eastern North America during the Pliocene to fill two niches: lacustrine piscivore (*S. namaycush*) and generalist (*S. fontinalis*), while *S. leucomaenis* diverged in the Pacific (Behnke 1972; Savvaitova 1980). These three species are well characterized (Behnke 1984, 1989).

Behnke (1984) divided *S.* [*Salvelinus*] in two species groups: the *S. malma* complex, containing the northern and southern forms of *S. malma*, *S. confluentus*, *S. leucomaenis*; and the *S. alpinus* complex. Species relationships within these two species groups are poorly understood because of a lack of comparative material throughout their distributions (Vladykov 1963; Behnke 1989).

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The common ancestor of *S. malma* and *S. alpinus* was widespread across northern Asia and North America before the Pleistocene, but glacial events resulted in the divergence of these two groups (McPhail 1961; Behnke 1972, 1980, 1984; Savvaitova 1980).

Salvelinus malma occurs on the eastern and western sides of the Pacific Ocean and exhibits diverse life histories, including anadromous and resident forms and partitioning of food resources within one habitat (Armstrong and Morrow 1980). However, *S. alpinus*, holarctic in distribution, shows the most phenotypic plasticity of all charrs. Landlocked and anadromous or dwarf and normal forms occupying several different feeding niches with different spawning times can occur sympatrically (Johnson 1980; Skulason et al. 1989; Snorrason et al. 1989; Svedang 1990), leading to debate over how many putative species may be incorporated into *S. alpinus*. Two models have been used to explain this apparent diversity: allopatric speciation before glaciation or sympatric speciation since glaciation, with most authors favoring the sympatric speciation model (Behnke 1972; Hammar 1984; Nyman 1984).

Savvaitova (1980) and Mednikov et al. (1980) argue, using morphological and DNA–DNA hybridization data respectively, that *S. malma* and *S. alpinus* are conspecific. Further, *S. confluentus* was, until recently, thought conspecific with *S. malma* (Morton 1970). However, morphological review of sympatric charrs in Siberia (Chereshnev 1982; Glubokovskiy and Chereshnev 1981) and Alaska (DeLacy and Morton 1943; McPhail 1961) and putative "inland *S. malma* forms" in North America (Cavender 1978; Haas and McPhail 1991) has provided evidence for species status of *S. alpinus*, *S. malma*, and *S. confluentus*. Nucleolar organizer region (NOR) location (Phillips et al. 1989), chromosomal (Cavender 1984), morphological (Cavender 1980), mitochondrial DNA (mtDNA) (Grewe et al. 1990), and ribosomal RNA (rDNA) (Phillips and Pleyte 1991; Pleyte et al. 1992) studies generally place *S. malma* and *S. alpinus* as sister taxa. Additionally, cytological (Cavender and Kimura 1989) and rDNA data (Phillips and Pleyte 1991; Phillips et al. 1992; Pleyte et. al. 1992) indicate a close relationship between *S. confluentus* and *S. leucomaenis*. Though placed in the subgenus *S.* [*Salvelinus*] by Behnke, *S. leucomaenis* may be more closely related to *S. namaycush* and *S. fontinalis* based on chromosomal (Phillips et al. 1989), rDNA (Phillips and Pleyte 1991), and morphological (Stearley 1993) data, supporting Savvaitova's (1980) hypothesis of a Pliocene separation of these taxa.

Genetic data often provide clear evidence to resolve systematic relationships, but this has not been the case with *Salvelinus*. Chromosome data (Cavender 1984; Phillips et al. 1989), mtDNA data (Ankenbrandt 1988; Grewe et al. 1990), rDNA data (Phillips and Pleyte 1991; Phillips et al. 1992; Pleyte et al. 1992), and allozyme data (Okazaki 1974; Johnson 1984) produce phylogenies with differing branching orders of the subgenera. Chromosomal data support species status of *S. malma*, *S. confluentus*, and *S. alpinus* within the subgenus *S.* [*Salvelinus*]. However, in mtDNA (Grewe et al. 1990) and DNA–DNA hybridization (Mednikov et al. 1980) studies, *S. alpinus* and *S. malma* did not fall into two clearly defined species groups.

Allelic variation of enzyme-encoding loci is a commonly used method of determining species and their relationships (Avise 1974; Buth 1984; Murphy et al. 1990). Hillis and Moritz (1990) recommend using protein electrophoresis for evaluating species relationships if the taxa in question have diverged in the past 5 million yr. Estimates of species divergence times in *Salvelinus* fall within this range (Grewe et al. 1990); therefore, allozyme variation should be able to resolve species relationships. Additionally, *Salvelinus* is of tetraploid origin and sampling a large number of loci is comparatively easy.

Protein electrophoresis of enzymes and blood proteins have been used to determine stock structure of *S. confluentus* (Leary et al. 1993), *S. malma* (Pustovoit 1991; Mitsuboshi et al. 1992), *S. fontinalis* (Stoneking et al. 1981), and *S. namaycush* (Ihssen et al. 1988; Krueger et al. 1989); to explore hybridization between *S. confluentus* and *S. fontinalis* (Leary et al. 1983) and between *S. alpinus* and *S. namaycush* (Wilson and Hebert 1993); to investigate genetic distinctions between *S. alpinus* and *S. malma* (Kartavstev et al. 1983; Gharret et al. 1991; Reist et al. 1995) and between *S. leucomaenis* and *S. malma* subspecies (Yoshiyasu and Humoto 1972; Yoshiyasu 1973); and to determine if biochemical differences exist between morphotypes and ecotypes of *S. alpinus* (e.g., Kornfield et al. 1981; Hindar et al. 1986; Magnusson and Ferguson 1987).

Despite the potential for allozyme data to contribute to our understanding of *Salvelinus* systematics, few electrophoretic studies have simultaneously examined charrs from Asia, Europe, and North America to determine species relationships. Okazaki (1974) studied the genetic relationships among Japanese salmonids. He sampled populations of *S. fontinalis*, *S. leucomaenis*, *S. l. pluvius*, *S. l. imbrius*, *S. malma*, *S. m. miyabe*, and *S. namaycush* but did not examine *S. alpinus*. Omel'chenko (1975), investigating the genetic relationships among Russian charrs, examined *S. fontinalis*, but did not include representatives of *S. namaycush* or North American *S. alpinus* and *S. malma*. Similarly, Johnson (1984) estimated the phylogeny of North American salmonids, including *S. alpinus*, *S. fontinalis*, and *S. namaycush*, from allele frequency data from 51 loci. However, he did not sample any populations of *S. malma* or *S. confluentus*.

Our objective in this study was to reexamine the evolutionary relationships among North American and Asian representatives of charrs using data from a large number of allozyme loci. Allozyme data from 60 loci support species status for *S. alpinus*, *S. malma*, and *S. confluentus* and indicate sister group relationships between the following two groups: *S. malma* – *S. alpinus* and *S. leucomaenis* – *S. confluentus*.

Methods

We obtained 422 specimens representing six species of *Salvelinus* from North America, Europe, and Asia (Table 1). Rainbow trout (*Oncorhynchus mykiss*) and hucho (*Hucho perryi*) were also collected and used as an allele mobility standard and as an outgroup, respectively. Samples were collected on dry or wet ice and stored at −80°C prior to analysis. Voucher specimens were preserved for all *Salvelinus* populations except *S. malma*, North Slope, Alaska; *S. alpinus*, Norway; and *S. namaycush*, Pennsylvania. These are currently in −20°C storage at Alaska Department of Fish and Game, Anchorage, AK.

Samples of muscle, liver, and eye were dissected from each specimen and placed in a culture tube. An equal volume

TABLE 1. Collection location and sample size of populations analyzed in this study. Latitude and longitude coordinates given for wild-caught collections.

Population	*N*	Source
(1) *Hucho perryi*	5	Japan, Nikko Hatchery, Hokkaido Prefecture source
Oncorhynchus mykiss	3	Idaho, Clear Springs Trout Company
(2) *Salvelinus alpinus*	12	Alaska, Clear Hatchery, Lake Aleknagik source
(3) *S. alpinus*	47	New Brunswick, Flower's Cove Hatchery, Walton Lake source
(4) *S. alpinus*	25	Overvatn, Salangen River, Norway, 68°58′N, 17°58′E
(5) *S. confluentus*	1	Hazard Creek, Idaho, 45°11′N, 116°18′W
(6) *S. confluentus*	13	Pinto Lake, Alberta, 52°07′N, 116°51′W
(7) *S. confluentus*	50	British Columbia, Hill Creek Hatchery, Arrow Lakes source
(8) *S. fontinalis*	10	Hazard Creek, Idaho, 45°11′N, 116°18′W
(9) *S. fontinalis*	4	New York, hatchery stock, source unknown
(10) *S. fontinalis*	24	New Brunswick, Flower's Cove Hatchery, Phillips Hatchery, Maine source
(11) *S. fontinalis*	49	Nova Scotia, McGowan Lake Fish Hatchery
(12) *S. leucomaenis*	5	Japan, Nikko Hatchery, Akan Lake, Hokkaido Prefecture source
(13) *S. l. pluvius*	5	Japan, Nikko Hatchery, Miyakawa River, Gifu Prefecture source
(14) *S. l. pluvius*	5	Japan, Nikko Hatchery, Nigata Prefecture source
(15) *S. malma*	6	Endicott Canal, North Slope, Alaska, 70°22′N, 148°22′W
(16) *S. malma*	26	Auke Creek, Alaska, 58°22′N, 134°38′W
(17) *S. malma*	50	Fish Creek, Alaska, 58°19′N, 134°35′W
(18) *S. malma*	49	Osprey Lake, Alaska, 56°24′N, 134°40′W
(19) *S. malma*	5	Japan, Nikko Hatchery, Niseko, Hokkaido Prefecture source
(20) *S. m. miyabe*	5	Japan, Nikko Hatchery, Lake Shikaribetsu, Hokkaido Prefecture source
(21) *S. namaycush*	6	New York, hatchery stock, Seneca strain
(22) *S. namaycush*	20	Pennsylvania, Allegheny National Fish Hatchery, Seneca strain
(23) *S. namaycush*	5	Japan, Nikko Hatchery

of a saturated pyridoxal-5-phosphate solution was added to the tissue, and samples were refrozen to lyse cells and free water-soluble proteins. Protein extracts were electrophoresed following the methods of May et al. (1979). Four buffer systems were used: (1) amine – citric acid buffer, pH 6.1 (Clayton and Tretiak 1972); (2) amine – citric acid buffer, pH 6.9 (Clayton and Tretiak 1972); (3) Tris – boric acid gel buffer, pH 8.7 lithium hydroxide – boric acid electrode buffer, pH 8.0 (Ridgway et al. 1970); and (4) Tris – boric acid – EDTA buffer, pH 8.7 (Boyer et al. 1963). Protein encoding loci were resolved using the recipes of Harris and Hopkinson (1976) and Allendorf et al. (1977). We used the genetic nomenclature of the American Fisheries Society (Shaklee et al. 1990). Alleles for each locus were referenced to the rainbow trout control (**100*) allele to facilitate future systematic studies within Salmonidae.

We scored duplicated locus phenotypes using the conventions of Allendorf (1975) along with available *Salvelinus* inheritance data. *sAAT-1,2** and *sMDH-B1,2** were scored as one locus with four allele doses (May et al. 1979; Johnson 1984). Three isolocus pairs were treated as if only one locus varied: *sIDHP-1,2** (all variation at *sIDHP-1**; May et al. 1979); *MEP-1,2** (all variation at *MEP-1**; Stoneking et al. 1979); and *TPI-1,2** (all variation at *TPI-1**). Both PEPD loci were variable, but a variant allele was assigned to one locus only (see Appendix 1).

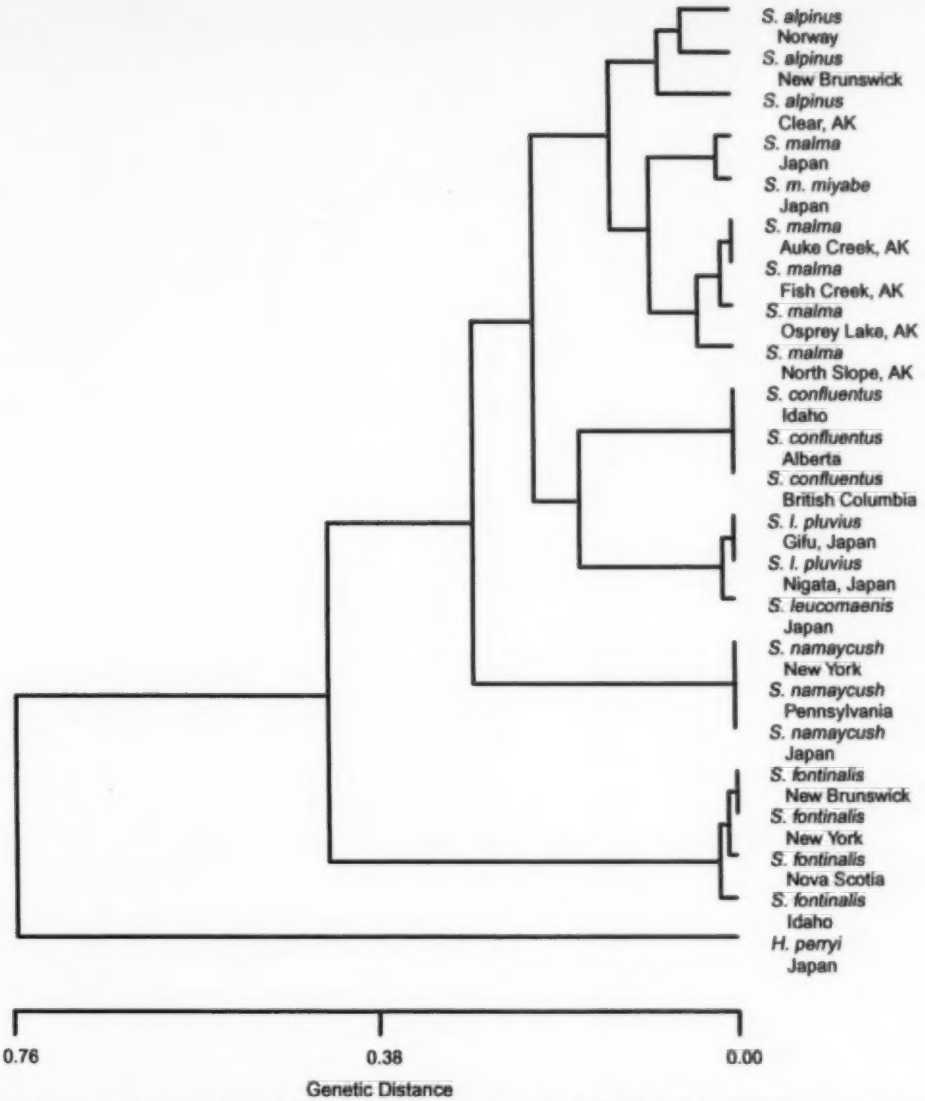

FIG. 1. UPGMA phenogram (cophenetic correlation = 0.982, percent standard deviation = 18.696), generated using Nei's (1978) unbiased genetic distance depicting genetic relationships among *Salvelinus* and *Hucho*.

We did not use available inheritance data to score FH variation. Marsden et al. (1987) proposes that FH variation in *S. namaycush* is a reflection of two isoloci based on family data. Though heterozygote phenotypes were clearly apparent at low frequencies in this study, resolution was not sufficient to repeatably score allele doses at *FH-1** or *FH-2**. Therefore, we conservatively scored FH phenotypes as if they were encoded by one locus with two allele doses. This should have little impact on the data analyses for two reasons. First, variant allele frequencies at *FH-1** were similar among polymorphic taxa. Second, frequency differences among taxa impact genetic distances less than do the presence or absence of alleles at a locus (Farris 1981).

We used BIOSYS-1 (Swofford and Selander 1981) to calculate Nei's (1978) unbiased distance and Cavalli-Sforza and Edwards' (1967) chord distance from allele frequencies. We generated a phenogram from Nei's unbiased distance using the Unweighted Pair Group Measures with Arithmetic Averaging algorithm (UPGMA; Sneath and Sokal 1973) in BIOSYS-1. UPGMA clusters taxa based on their overall similarity. The Fitch and Margoliash algorithm (FM; Fitch and Margoliash 1967), which fits branch lengths to observed distance data using a least-squares criterion, was used to construct a tree with Cavalli-Sforza and Edwards' (1967) chord distance with the global branch swapping option in PHYLIP version 3.3 (Felsenstein 1990).

To avoid introducing error by converting allele frequencies into one number (Hillis 1987), we used the CONTML routine with the global branch swapping option of PHYLIP. This program square root transforms allele frequency data and builds a tree that maximizes the likelihood of observed gene frequencies.

TABLE 2. Tissues (M = muscle, L = liver, E = eye) and buffer systems used to resolve enzyme coding loci. Enzyme names follow IUBNC (1984).

Enzyme	IUBNC No.	Locus	Tissue	Buffer[a]
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	M	2
		*sAAT-3**	E	1, 3
		*sAAT-4**	L	1, 3
Adenosine deaminase	3.5.4.4	*ADA-1**	M	4
		*ADA-2**	M	4
Alcohol dehydrogenase	1.1.1.1	*ADH**	L	3
Adenylate kinase	2.7.4.3	*AK-1**	M	1
		*AK-2**	M	1
Alanine aminotransferase	2.6.1.2	*ALAT**	M	4
Creatine kinase	2.7.3.2	*CK-A1**	M	3
		*CK-A2**	M	3
		*CK-B**	E	3
Esterase	3.1.1.1	*EST-1**	L	3
		*EST-2**	L, M	3
		*EST-3**	L	3
		*EST-4**	L	3
Fructose-biphosphate aldolase	4.1.2.13	*FBALD-1**	M	2
		*FBALD-4**	E	2
Fumarate hydratase	4.2.1.2	*FH**	F	1
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	M	3
		*G3PDH-3**	M	3
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B1**	M	3
		*GPI-B2**	M	3
beta-Glucuronidase	3.2.1.31	*GUS**	L	4
L-Iditol dehydrogenase	1.1.1.14	*IDDH**	L	3
Isocitrate dehydrogenase	1.1.1.42	*mIDHP-1**	M	1
		*mIDHP-2**	M	1
		*sIDHP-1,2**	L	1
Lactate dehydrogenase	1.1.1.27	*LDH-A1**	M	3
		*LDH-A2**	M	3
		*LDH-B1**	M, E	3
		*LDH-B2**	L	3
		*LDH-C**	E	3
Malate dehydrogenase	1.1.1.39	*sMDH-A1,2**	L	1
		*sMDH-B1,2**	M	1
		*mMDH**	M	1
Malic enzyme	1.1.1.40	*MEP-1,2**	M, L	2
		*MEP-3**	L	2
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	L	2
Dipeptidase	3.4._._	*PEPA-1**	M	4
		*PEPA-2**	E	4
Tripeptide aminopeptidase	3.4._._	*PEPB-1**	M	4
		*PEPB-2**	E, M	4

TABLE 2. (*Concluded*)

Enzyme	IUBNC No.	Locus	Tissue	Buffer[a]
Proline dipeptidase	3.4.13.9	*PEPD-1,2**	M, E	2
Peptidase	3.4._._	*PEP-LT**	M, E	4
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	L	2
Phosphoglycerate kinase	2.7.2.3	*PGK-1**	M	1
Phosphoglucomutase	2.7.5.1	*PGM-1**	M	1
		*PGM-2**	M	1
Purine-nucleoside phosphorylase	2.4.2.1	*PNP-2**	E	4
Superoxide dismutase	1.15.1.1	*SOD**	L	4
Triosephosphate isomerase	5.3.1.1	*TPI-1,2**	M	4
		*TPI-3**	M	4

[a]1 = Amine – citric acid buffer, pH 6.1, 2 = amine – citric acid buffer, pH 6.9, 3 = tris – citric acid gel buffer, lithium hydroxide electrode buffer, pH 8.5, 4 = Tris – boric acid – EDTA buffer, pH 8.5.

Because equal rates of evolution among lineages is an assumption of the UPGMA algorithm, we performed an *F*-ratio test (Felsenstein 1984) using the formulae of Sheldon (1987) to calculate the *F* statistic and degrees of freedom. This test compares the sums of squares (the difference between observed and expected distances averaged over all tree branches) of trees generated by the FM and KITCH algorithms in PHYLIP. The FM algorithm has no assumption of equal rates of evolution, while the KITCH algorithm constrains branch lengths according to a molecular clock. The null hypothesis of equal rates among lineages is rejected if the FM tree has a statistically "better fit" than the KITCH tree.

We evaluated all phylogenetic estimates using two methods: parsimony and likelihood. Total tree lengths were calculated using the USERTREE option of FREQPARS (Swofford and Berlocher 1987), which fits branch lengths to input tree topologies. The CONTML program of PHYLIP was used to perform *Z* tests ($\alpha = 0.10$) among likelihoods of input tree topologies to determine if any input tree is statistically more likely than another.

We tested the FM tree for internal inconsistencies by jackknifing and built a Jackknife Strict Consensus (JSC) tree (Lanyon 1985). Jackknifing indirectly estimates variance of branching points by removing one ingroup taxon at a time and rebuilding the tree. Nodes that are poorly supported due to inconsistencies in the data collapse when a taxon is removed. A JSC tree is built from the points on which all pseudoreplicate trees agree.

Results

Gene products from 60 presumptive electrophoretic loci were resolved from muscle, liver, and eye tissues (Table 2). We focused our reporting of genetic data on allelic frequency differences observed among *S. alpinus*, *S. confluentus*, *S. leucomaenis*, and *S. malma*. *Salvelinus fontinalis* and *S. namaycush* are well established species and have been studied extensively for allozyme variation among populations.

No variation was detected at *sAAT-3**, *CK-A1**, *CK-B**, *EST-1**, *FBALD-1**, *FBALD-4**, *GPI-B1**, *GPI-B2**, *G3PDH-3**, *mMDH**, *PEPB-2**, *PGK-1**, *TPI-2**, and *TPI-3** among *Salvelinus* taxa examined. Allele frequency data for polymorphic loci are presented in Appendix 1. *Salvelinus confluentus* exhibited unique alleles at *MPI** and *PEPD-2**. All three populations of *S. confluentus* were homozygous for *ADA-1*125*. This allele only occurred in one *Salvelinus* population examined, *S. malma*, Osprey Lake, at a frequency of 0.01. At *LDH-A2**, *S. l. leucomaenis* and *S. l. pluvius* allozymes had similar mobility but reduced staining intensity from other *Salvelinus*. This phenomenon was uniform and repeatable, indicating different kinetic properties of *S. l. leucomaenis* and *S. l. pluvius* *88* allozymes, and was therefore scored as a unique allele (*88′).

All populations of *S. alpinus* were fixed for different alleles at *AK-2** and *PGM-1** from all populations of *S. malma*. North American *S. malma* expressed a diagnostic allele, *SOD*152*, not seen in any population of *S. alpinus*. *Salvelinus alpinus* from Clear, Alaska, were fixed for a different allele at *PGM-2** than were all other populations of *S. alpinus*. This allele was seen at a low frequency (0.10) in one North American *S. malma* population. Additionally, a unique allele was observed at *sAAT-1,2** in *S. alpinus* from New Brunswick. All *S. alpinus* populations shared a common *PEPD-1** and *EST-2** allele with Japanese *S. m. malma* and *S. m. miyabe*. Japanese *S. m. malma* and *S. m. miyabe* shared the **200* allele at *SOD** with *S. confluentus*, *S. l. leucomaenis*, and *S. l. pluvius*, differing from all populations of *S. alpinus* and North American *S. malma*.

Nei's (1978) unbiased distances ranged within species from 0.000 (*S. confluentus*, *S. fontinalis*, *S. malma*, and *S. namaycush* populations) to 0.100 (*S. malma* populations) and between species from 0.104 (*S. alpinus* Clear, Alaska, and *S. malma*, Japan) to 0.493 (*S. alpinus*, Clear, Alaska and *S. fontinalis*, Nova Scotia). Cavalli-Sforza and Edwards' (1967) chord distances ranged within species from 0.016 (*S. confluentus*) to 0.285 (*S. malma*) and between species from 0.294 (*S. malma*, Japan, and *S. alpinus*, Clear, Alaska) to 0.564 (*S. alpinus*, Clear, Alaska, and *S. fontinalis*, New Brunswick).

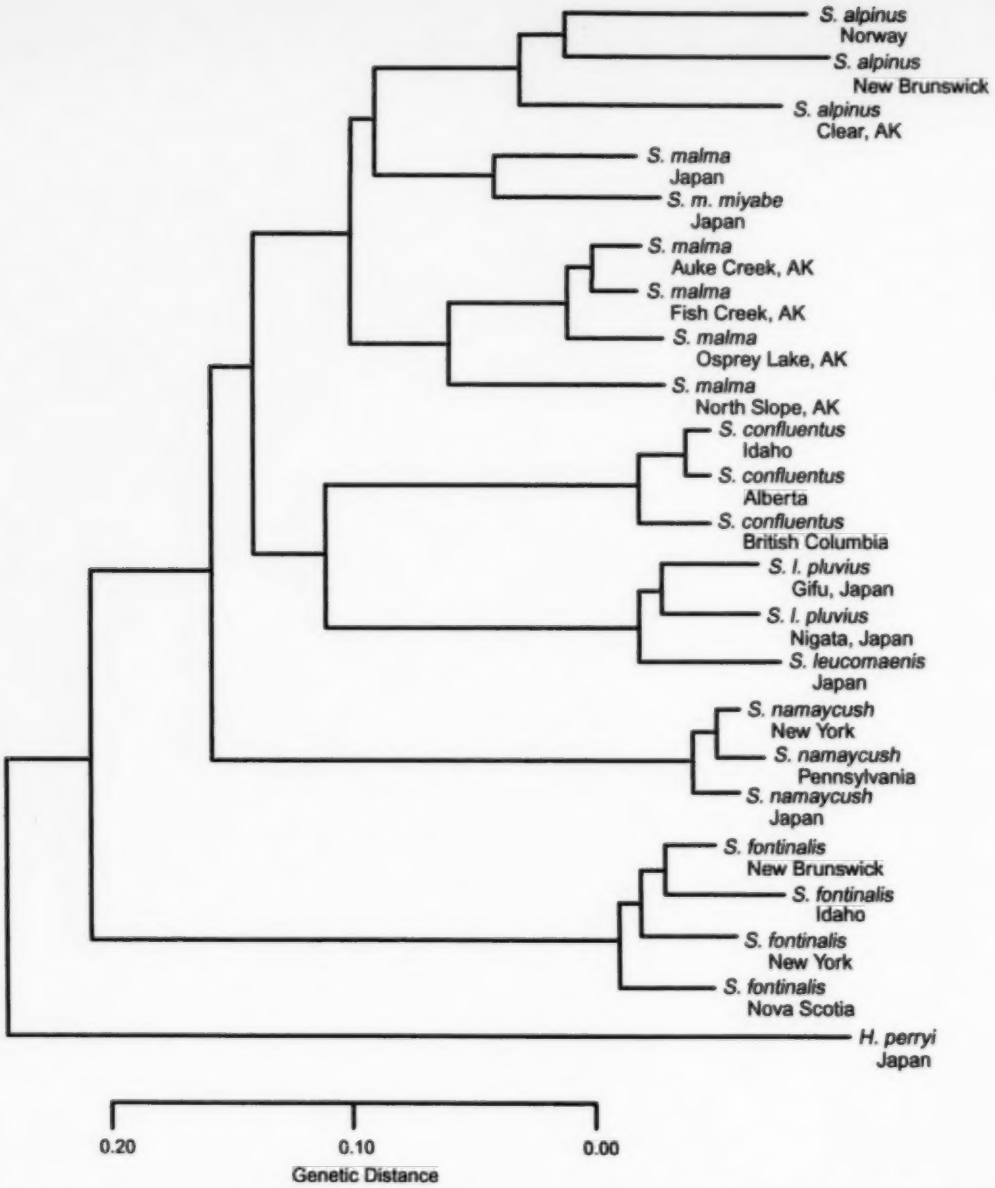

FIG. 2. Fitch and Margoliash least-squares tree (percent standard deviation = 3.650, 1921 trees examined) derived using Cavalli-Sforza and Edwards' (1967) genetic distance.

The UPGMA tree (Fig. 1), FM tree (Fig. 2), and the ML tree had very similar topologies. In all trees, *S. fontinalis* and *S. namaycush* represented the most divergent lineages. *Salvelinus l. leucomaenis* and *S. l. pluvius* were the sister group to *S. confluentus*; these formed the next most divergent lineage. *Salvelinus alpinus* and *S. malma* were sister taxa, and were the last groups to branch.

Conspecific populations grouped together with one exception. In the UPGMA tree, Japanese *S. m. malma* and *S. m. miyabe* formed their own unit and were sister group to North American S. malma. In the FM and ML tree, Japanese *S. malma* were the sister group to *S. alpinus*. The FM and ML trees were virtually identical. However, the ML tree grouped *S. malma* from Osprey Lake and Fish Creek, Alaska and grouped *S. l. leucomaenis* with *S. l. pluvius*, Nigata.

The UPGMA algorithm assumes rates of evolution are the same among all lineages. The validity of applying a molecular clock hypothesis to these *Salvelinus* allozyme data was evaluated using the *F*-ratio test (Felsenstein 1984). The FM tree had a significantly better fit ($F = 32.13$, df(KITCH) = 231, df(FM) = 210, $P < 0.01$). Therefore rate variation exists among *Salvelinus* lineages.

Total tree lengths calculated by FREQPARS showed that the FM tree topology required less changes than the other

solutions (Table 3). No topology was significantly more likely than any other (Table 3).

Lanyon's jackknife (1985) was applied to detect areas of uncertainty in the FM tree. An FM tree was rebuilt for each of 22 pseudoreplicates and a JSC tree (Fig. 3) was built from all pseudoreplicate trees. This analysis detected one area of instability: the node grouping Japanese *S. m. malma* and *S. m. miyabe* with the *S. alpinus* clade collapsed when each of these taxa was deleted from the analysis.

Discussion

Selection of the Best Phylogenetic Estimate

Felsenstein (1984) argues that phylogenetic inference is a matter of statistics; Sober (1988) continues this argument and states that a process model underlying variation of the data at hand should approximate the assumptions of the inferential method. Two models, the infinite alleles model and random genetic drift have been used to develop systematic analysis techniques. Which of these models can best explain the variation in *Salvelinus* allele frequencies?

The infinite alleles model (Kimura 1968) states that the number of alleles in a population is kept constant by a balance of drift eliminating alleles and mutation creating new alleles. Distances estimated using this model assume equal effective population sizes among lineages; distances estimated using a random drift only model do not (Felsenstein 1985). Because *Salvelinus* are among the most northerly dwelling species where Pleistocene glaciation events were a dominant speciation force (McPhail 1961; Behnke 1972; Briggs 1986), equal effective population sizes may not be a valid assumption. Genetic drift plays a significant role in contributing to allelic differences among small isolated populations (e.g., Soule 1976). Additionally, Hindar (1986) argues that drift is a more likely explanation for observed genetic differences among *S. alpinus* populations than a previously proposed mutation–selection model.

Given a model of pure genetic drift, Felsenstein (1985) recommends using Cavalli-Sforza and Edwards' (1967) chord distance with an appropriate clustering algorithm or using a maximum likelihood technique with observed gene frequencies (Felsenstein 1981). Cavalli-Sforza and Edwards' distance assumes drift only is responsible for allele frequency changes (Swofford and Olsen 1990). The maximum likelihood algorithm uses a model that is intended to simulate random drift (Felsenstein 1981). The FM phylogeny calculated from Cavalli-Sforza and Edwards' distance and the ML tree were derived from models that we believed incorporated the best model underlying *Salvelinus* genetic variation, drift.

Another consideration in choosing an appropriate analytical technique is the applicability of a molecular clock. Some algorithms (UPGMA, KITCH routine in PHYLIP) assume that rates of evolution are the same among all lineages. Hillis (1985, p. 118) suggests that "...a molecular clock should not be an *a priori* assumption in electrophoretic studies." Because rates of evolution among lineages varied in *Salvelinus* and Nei's (1978) genetic distance assumes an infinite alleles model, this analysis may not have had as much potential to accurately reflect phylogenetic relationships among *Salvelinus* as other methods.

Two different point estimates of *Salvelinus* phylogeny were produced by the most appropriate data analysis techniques (FM and ML). We selected the FM topology as our best phylogenetic estimate based on two criteria: parsimony and evidence of gene flow. The FM algorithm produced a shorter tree. The shortest tree accounts for the smallest number of allele frequency changes between taxa and, therefore, has the least amount of homoplasy (Farris 1983).

TABLE 3. Tree topologies representing three different estimates of evolutionary relationships among species in the genus *Salvelinus* were evaluated by the following methods. Tree lengths were calculated using the USERTREE option of FREQPARS (Swofford and Berlocher 1987). Likelihoods were derived from the CONTML routine of PHYLIP (Felsenstein 1990).

Algorithm used to generate topology	Tree length	Likelihood
UPGMA, Nei's (1978) genetic distance	198.741	4547.540
Maximum likelihood	196.661	4564.429
Fitch and Margoliash, Cavalli-Sforza and Edwards' (1967) genetic distance	196.600	4558.492

Though the FM tree was not significantly more likely than the ML tree, it was biologically more meaningful. These trees differed in that the FM tree grouped Auke Creek, Alaska, and Fish Creek, Alaska, *S. malma*, while the ML tree grouped Fish Creek, Alaska, and Osprey Lake, Alaska, *S. malma*. Auke Creek and Fish Creek are streams located within 10 km of each other near Juneau, Alaska. Osprey Lake is a stocked system on Baranof Island in southeast Alaska, where natural immigration is unlikely to occur, though outmigration can occur. Heterogeneity analyses were performed between these three populations. No significant heterogeneity was found between Auke and Fish Creek *S. malma* ($\chi^2 = 18.63$, df = 12, $P = 0.10$), whereas significant heterogeneity did exist between Auke Creek and Osprey Lake *S. malma* ($\chi^2 = 72.14$, df = 14, $P < 0.01$) and between Fish Creek and Osprey Lake *S. malma* ($\chi^2 = 90.94$, df = 13, $P < 0.01$).

The best phylogenetic hypothesis was the FM topology. The assumptions of this reconstruction technique are the most appropriate for these data, it produced the most parsimonious tree, and the topology was the most biologically meaningful.

Phylogenetic Hypotheses

We jackknifed the FM topology to indirectly estimate the confidence of the tree (Lanyon 1985). The exact statistical properties of jackknifing are not understood, i.e., the frequency with which a node collapses cannot be correlated with the reliability of that node (Felsenstein 1988), but jackknifing can successfully locate areas of instability (Pamilo 1990). It is not surprising that the node connecting Japanese *S. malma* and *S. alpinus* collapsed. The likelihood analysis demonstrated that it is equally likely that Japanese *S. malma* cluster with *S. alpinus* as with North American *S. malma*.

The JSC tree revealed the following relationships within the genus *Salvelinus* with some degree of confidence. The compositions and branching order of the subgenera *S.* [*Baione*] (*S. fontinalis*), *S.* [*Cristivomer*] (*S. namaycush*),

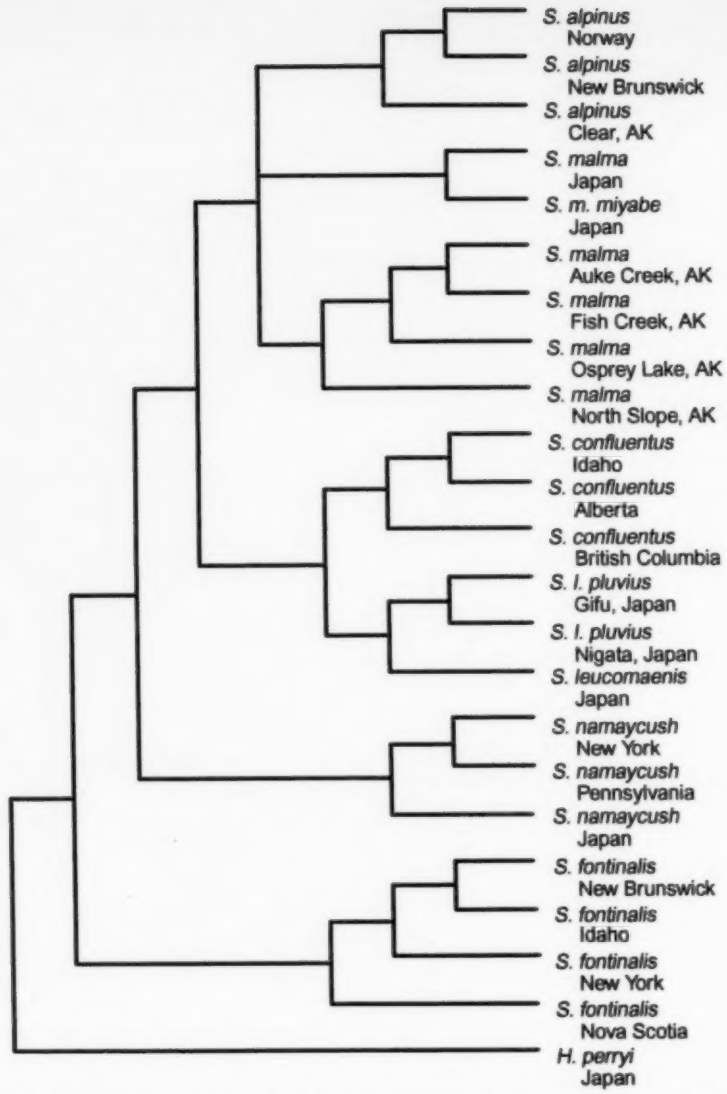

FIG. 3. Jackknife Strict Consensus tree built from 22 pseudoreplicate Fitch and Margoliash trees.

and *S.* [*Salvelinus*] (*S. malma* and *alpinus* complexes), were clearly resolved. *S.* [*Baione*] was the most divergent lineage followed by *S.* [*Cristivomer*]. This conflicted with morphological (Stearley 1993) and some mitochondrial data (Ankenbrandt 1988), which suggest that *S.* [*Salvelinus*] is the most divergent lineage and *S.* [*Cristivomer*] the least evolved. It also conflicted with rDNA data (Phillips et al. 1992; Pleyte et al. 1992), which show *S. confluentus* and *S. leucomaenis* as the most divergent lineages. However, it concurred with the hypothesis of evolutionary relationships within the genus *Salvelinus* presented by Behnke (1972) and supported chromosomal (Cavender 1984; Phillips et al. 1989) and previous allozyme data (Okazaki 1974; Johnson 1984). The mitochondrial data of Grewe et al. (1990) also suggest this relationship.

An important aspect of this present study was the broad geographical distribution of populations sampled within the subgenus *S.* [*Salvelinus*]. The JSC tree revealed two sister group relationships in this subgenus: *S. leucomaenis* – *S. confluentus* and *S. alpinus* – *S. malma*. However, within the *S. alpinus* – *S. malma* species complexes, *S. alpinus* and *S. malma* did not separate in two monophyletic groups; there is an unresolved trichotomy among Asian *S. malma*, North American *S. malma*, and *S. alpinus*.

Our findings supported the hypothesis that *S. leucomaenis* and *S. confluentus* are sister species. The placement of both these taxa has always been controversial. Though provisionally allied with the *S. malma* complex (Behnke 1980, 1984, 1989), *S. leucomaenis* has also been grouped with *S. namaycush* and *S. fontinalis* based on chromosomal and

morphological data (Savvaitova 1980; Stearley 1993). However, chromosome data indicate that *S. leucomaenis* and *S. confluentus* have intermediate karyotypes between the primitive karyotypes of *S. fontinalis* and *S. namaycush* and the advanced karyotypes of *S. alpinus* and *S. malma* (Cavender 1980; Phillips et al. 1989). Additionally, rDNA data (Phillips and Pleyte 1991; Phillips et al. 1992; Pleyte et al. 1992) indicate a sister relationship between *S. leucomaenis* and *S. confluentus*. More populations of these two species will need to be studied to further test the sister group hypothesis. These should include *S. confluentus* from the Columbia River basin where this species is thought to have invaded North America (Behnke 1979) and perhaps representatives of *S. albus* from Kamchatka, a possible Asian relative of *S. confluentus* (Behnke 1980, 1984).

North American *S. malma*, Japanese *S. malma*, and *S. alpinus* remained an unresolved trichotomy in this study. What can be said about species distinctions between *S. malma* and *S. alpinus* based on allozyme data? Leary and Booke (1990) stress the importance of looking at the number of loci where complete divergence exists to determine species distinctions, not genetic distances and phylogenetic trees estimated from them. If two populations do not share any common alleles at several loci, gene flow between the populations is not occurring and reproductive isolation exists. Distances cannot as easily be used as evidence of gene flow.

In North America, a species level distinction between these two taxa was first described by DeLacy and Morton (1941) through a morphological examination of sympatric charrs in Karluk Lake, Alaska. Two forms in this lake exhibit nonoverlapping character states and no evidence of hybridization. Morphological delineation of these two species was refined by McPhail (1961) and Morrow (1980) in Alaska and Chereshnev (1982) in Russia. Lack of gene flow between *S. alpinus* and *S. malma* has clearly been documented in three protein electrophoresis studies: Kartavstev et al. (1983) found a fixed difference at *ACPH** between sympatric *S. alpinus* and *S. malma* in the Chukchi; Gharrett et al. (1991) found a fixed difference at *sAAT-4** and *PEP-LL** (resolved with leucyl-leucine) between sympatric *S. alpinus* and *S. malma* in Karluk Lake, Alaska; and Reist et al. (1995) found fixed differences at *PGM-1** between Alaskan stocks of *S. alpinus* and *S. malma*. Additionally, our data also showed *S. malma* and *S. alpinus* to be well defined species owing to fixed differences at *AK-2** and *PGM-2**. It is interesting to note that the loci described in these studies can be used as evidence of a lack of gene flow between species, but may not necessarily be diagnostic loci for the two species throughout their ranges.

McPhail (1961) extensively examined the charrs of Alaska. Two forms of *S. alpinus* are described based on gill raker and vertebrae counts: a western Arctic–Bering Sea form distributed from the Kuskokwim River to the Seward Peninsula, and an eastern form distributed from the Kuskokwim River south. He also describes a northern and southern form of *S. malma*, also based on gill raker and vertebrae counts. McPhail postulates that northern and southern forms of *S. malma* were originally a single population. Glaciation separated the populations, and the northern form differentiated off the coast of Siberia and Alaska, while the southern form differentiated further south. The northern form is distributed from the Alaska Peninsula to the Mackenzie River in North America and in Asia in Okhotsk Sea drainages north to the Chukotsk Peninsula. The southern form ranges in North America from the Alaska Peninsula south to northern California and in Asia from the Amur River south to the Sea of Japan.

Morrow (1980) commented on the morphological and ecological similarity of the western Arctic – Bering Sea *S. alpinus* (first described by McPhail 1961) and the northern form of *S. malma*. Discriminant function analysis of morphological data consistently groups this putative form of *S. alpinus* with the northern form of *S. malma* (Morrow 1980). A recent meristic and allozyme analysis of unknown charrs on the North Slope of Alaska, compared with known *S. malma* and *S. alpinus* populations (Reist et al. 1995) provides genetic evidence to support Morrow's hypothesis. Reist et al. found one locus (*PGM-1**) with a fixed difference between known *S. alpinus* and *S. malma* populations. North Slope charr were essentially fixed for *S. malma* alleles at this locus.

We felt that these data show the possibility of remnant symplesiomorphic alleles remaining in North Slope *S. malma* populations. Our data further supported the use of *S. malma* to describe North Slope charr with the addition of another diagnostic locus. Our data also indicated potential retention of symplesiomorphic alleles in North Slope *S. malma*; identical variant *sAAT-1,2** alleles were seen in North Slope charr and Clear, Alaska, *S. alpinus*. All other populations of *S. malma* examined were fixed for one allele at *sAAT-1,2**.

Our allozyme data also suggested a genetic separation of northern and southern forms of North American *S. malma*, named *S. m. malma* and *S. m. lordi* respectively by Behnke (1984). A fixed difference at *PEP-LT** occurred between *S. malma* from the North Slope, Alaska (northern form), and *S. malma* from Osprey Lake, Fish Creek, and Auke Creek, Alaska.

The inability to locate Japanese *S. malma* in the phylogeny was not surprising. Previous morphological studies (see Behnke 1984 for review) and cytological studies (Cavender and Kimura 1989) show that charr from Japan express characters similar to the northern and southern forms of *S. malma* with no specific affinity to either form. Additionally, *S. m. miyabe*, a charr found only in Lake Shikaribetsu in Japan, differs from *S. m. malma* in meristic, morphometric, and color characteristics (Maekawa 1984) and shares a common number of vertebrae and gillrakers with *S. alpinus*. Though genetically similar to *S. malma* (Mitsoboshi et al. 1992), it has also been called a relict population of *S. alpinus* (Behnke 1972, 1984) based on morphology. Our allozyme data also supported the subspecific status of a southern Asian *S. malma* form, termed *S. m. krascheninnikovi* by Behnke (1984, 1989). However, it did not aid in aligning this charr with either the northern or southern form of *S. malma*. In fact, these populations shared alleles with *S. alpinus* at peptidase and esterase loci. This may be because of incomplete fixation for *S. malma* alleles or an example of convergent evolution. Additionally, southern Asian *S. malma* have higher gill raker and vertebrae counts than their North American counterparts. Behnke (1980) suggests this may be due to introgression with the northern form of *S. malma*. Perhaps it is due to introgression with *S. alpinus*, possibly explaining the presence of *S. alpinus* alleles at two loci in these taxa.

Behnke (1984, 1989) separates the *S. alpinus* complex into five subspecies: *S. a. alpinus* distributed in Great Britain

and Scandinavia; *S. a. salvelinus* found in isolated lakes in central Europe; *S. a. erythrinus* in Siberia; *S. a. stagnalis* in northern North America; *S. a. oquassa*, glacial relict populations in northeastern North America; and *S. a. taranetzi* in southwest Alaska, Kamchatka, and the Chukotsk Peninsula. This study also supported the subspecific status of these charrs and indicated a close genetic relationship between *S. a. oquassa* (New Brunswick samples) and *S. a. alpinus* (Norway samples). Behnke (1984) postulates a European origin for these two charrs, and an Asian origin for *S. a. erythrinus* and *S. a. taranetzi*.

Because evolution occurs at the population level and not at the species level (Nelson 1989), systematic relationships within a group can only be resolved if the entire range of that group is sampled. Therefore, we electrophoretically examined *Salvelinus* populations over a wide geographic distribution. Because of the broad range of sampling locations, the branching order of the subgenera *S.* [*Baione*], *S.* [*Cristivomer*], and *S.* [*Salvelinus*] was resolved as well as confirming the sister group relationship between *S. leucomaenis* and *S. confluentus* and between *S. malma* and *S. alpinus*. To determine the mechanisms allowing divergence within the *S. alpinus* – *S. malma* complex more completely, more populations will need to be studied, including *S. alpinus* and *S. malma* from Asia and *S. malma* populations from more southerly locations in North America.

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APPENDIX. Allele frequency estimates for polymorphic loci in *Hucho* and *Salvelinus* populations examined. Numbers following a species name correspond with sampling locations in Table 1. No or reduced isozyme activity at a particular locus was scored as a unique allele. This is indicated by an asterisk marked with a prime (*').

Locus	sAAT-1,3*							sAAT-4*					ADA-1*						ADA-2*					ADH*		AK-1*			AK-2*		
Population	(N)	*100	*47	*70	*84	*60	*97	(N)	*100	*22	*166	*388	(N)	*100	*88	*118	*126	*136	(N)	*100	*109	*104	*77	(N)	*-100	*-170	(N)	*100	*38	(N)	*100	*-400
H. perryi (1)	5	0.000	1.000	0.000	0.000	0.000	0.000	5	0.000	0.000	0.000	1.000	5	0.000	1.000	0.000	0.000	0.000	5	0.000	0.000	0.000	1.000	5	0.000	1.000	5	1.000	0.000	5	1.000	0.000
S. alpinus (2)	12	0.000	0.000	0.583	0.000	0.417	0.000	12	0.083	0.917	0.000	0.000	12	0.000	0.000	0.000	0.000	1.000	12	0.000	1.000	0.000	0.000	12	1.000	0.000	12	1.000	0.000	12	0.000	1.000
S. alpinus (3)	47	0.000	0.000	0.755	0.043	0.000	0.202	42	0.179	0.821	0.000	0.000	47	0.000	0.000	0.000	0.000	1.000	47	0.000	1.000	0.000	0.000	47	1.000	0.000	47	1.000	0.000	47	0.000	1.000
S. alpinus (4)	25	0.000	0.000	1.000	0.000	0.000	0.000	25	1.000	0.000	0.000	0.000	25	0.000	0.000	0.000	0.000	1.000	25	0.000	1.000	0.000	0.000	24	1.000	0.000	25	1.000	0.000	25	0.000	1.000
S. confluentus (6)	1	0.000	0.000	1.000	0.000	0.000	0.000	1	0.000	1.000	0.000	0.000	1	0.000	0.000	0.000	1.000	0.000	1	0.000	1.000	0.000	0.000	1	1.000	0.000	1	1.000	0.000	1	1.000	0.000
S. confluentus (6)	13	0.000	0.000	1.000	0.000	0.000	0.000	8	0.000	1.000	0.000	0.000	13	0.000	0.000	0.000	1.000	0.000	13	0.000	1.000	0.000	0.000	12	1.000	0.000	13	1.000	0.000	13	1.000	0.000
S. confluentus (7)	50	0.000	0.000	1.000	0.000	0.000	0.000	50	0.000	1.000	0.000	0.000	50	0.000	0.000	0.000	1.000	0.000	50	0.000	1.000	0.000	0.000	50	1.000	0.000	50	1.000	0.000	50	1.000	0.000
S. fontinalis (8)	10	0.000	0.000	0.850	0.150	0.000	0.000	8	0.313	0.000	0.688	0.000	10	0.000	0.000	1.000	0.000	0.000	10	0.000	0.950	0.050	0.000	10	1.000	0.000	10	1.000	0.000	10	1.000	0.000
S. fontinalis (9)	4	0.000	0.000	0.750	0.125	0.125	0.000	4	1.000	0.000	0.000	0.000	4	0.000	0.000	1.000	0.000	0.000	4	0.000	1.000	0.000	0.000	4	0.875	0.125	4	1.000	0.000	4	1.000	0.000
S. fontinalis (10)	24	0.000	0.000	0.813	0.187	0.021	0.000	24	0.708	0.000	0.292	0.000	24	0.000	0.000	1.000	0.000	0.000	24	0.000	1.000	0.000	0.000	24	0.938	0.063	24	0.958	0.042	24	1.000	0.000
S. fontinalis (11)	49	0.000	0.000	0.898	0.041	0.061	0.000	49	1.000	0.000	0.000	0.000	49	0.000	0.000	1.000	0.000	0.000	49	0.000	1.000	0.000	0.000	49	1.000	0.000	49	1.000	0.000	49	1.000	0.000
S. leucomaenis (12)	5	0.000	0.000	1.000	0.000	0.000	0.000	5	0.000	0.800	0.400	0.000	5	0.000	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	5	1.000	0.000	5	1.000	0.000
S. l. pluvius (13)	5	0.000	0.000	1.000	0.000	0.000	0.000	5	0.000	0.900	0.100	0.000	5	0.000	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	5	1.000	0.000	5	1.000	0.000
S. l. pluvius (14)	5	0.000	0.000	1.000	0.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	0.000	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	5	1.000	0.000	5	1.000	0.000
S. malma (16)	8	0.000	0.000	0.917	0.000	0.083	0.000	8	1.000	0.000	0.000	0.000	8	0.000	0.000	0.000	0.000	1.000	8	0.000	1.000	0.000	0.000	8	1.000	0.000	8	1.000	0.000	8	1.000	0.000
S. malma (16)	28	0.000	0.000	1.000	0.000	0.000	0.000	28	0.875	0.125	0.000	0.000	28	0.000	0.000	0.000	0.000	1.000	28	0.000	1.000	0.000	0.000	28	1.000	0.000	28	1.000	0.000	28	1.000	0.000
S. malma (17)	50	0.000	0.000	1.000	0.000	0.000	0.000	48	0.885	0.115	0.000	0.000	50	0.000	0.000	0.000	0.000	1.000	50	0.000	1.000	0.000	0.000	50	1.000	0.000	50	1.000	0.000	50	1.000	0.000
S. malma (18)	49	0.000	0.000	1.000	0.000	0.000	0.000	49	0.939	0.061	0.000	0.000	49	0.000	0.000	0.000	0.010	0.990	49	0.000	1.000	0.000	0.000	49	1.000	0.000	49	1.000	0.000	49	1.000	0.000
S. malma (19)	5	0.000	0.000	1.000	0.000	0.000	0.000	5	0.500	0.500	0.000	0.000	5	0.000	0.000	0.000	0.000	1.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	5	1.000	0.000	5	1.000	0.000
S. m. miyabe (20)	5	0.000	0.000	1.000	0.000	0.000	0.000	5	0.500	0.500	0.000	0.000	5	0.000	0.000	0.500	0.000	0.500	5	0.000	1.000	0.000	0.000	5	1.000	0.000	5	1.000	0.000	5	1.000	0.000
S. namayoush (21)	8	0.000	0.000	0.417	0.583	0.000	0.000	8	1.000	0.000	0.000	0.000	8	0.000	0.000	0.000	0.500	1.000	8	0.000	1.000	0.000	0.000	8	1.000	0.000	8	1.000	0.000	8	1.000	0.000
S. namayoush (22)	20	0.000	0.000	0.350	0.650	0.000	0.000	18	1.000	0.000	0.000	0.000	20	0.000	0.000	0.000	0.000	1.000	20	0.000	1.000	0.000	0.000	18	1.000	0.000	20	1.000	0.000	20	1.000	0.000
S. namayoush (23)	5	0.000	0.000	0.400	0.600	0.000	0.000	5	1.000	0.000	0.000	0.000	5	0.000	0.000	0.000	0.000	1.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	5	1.000	0.000	5	1.000	0.000

Locus	ALAT*						CK-A1*				EST-2*					EST-3*			EST-4*					PH*			GAPDH-1*					GUS*		
Population	(N)	*100	*82	*84	*89	*83	(N)	*100	*80	*'	(N)	*100	*92	*97	*91	(N)	*100	*96	(N)	*100	*108	*97	*94	(N)	*100	*78	*72	(N)	*-100	*-60	*100	*-40	(N)	*100	*83
H. perryi (1)	5	0.000	0.000	0.000	1.000	0.000	5	0.000	0.000	1.000	5	0.000	0.000	0.000	1.000	5	0.000	1.000	5	0.000	0.000	1.000	0.000	5	0.000	0.000	1.000	5	0.000	0.000	0.000	1.000	5	1.000	0.000
S. alpinus (2)	12	0.000	0.000	1.000	0.000	0.000	12	0.000	1.000	0.000	12	0.000	0.000	1.000	0.000	12	0.708	0.292	12	0.000	1.000	0.000	0.000	12	1.000	0.000	0.000	12	0.000	1.000	0.000	0.000	12	1.000	0.000
S. alpinus (3)	47	0.000	0.000	1.000	0.000	0.000	47	0.000	1.000	0.000	47	0.000	0.000	1.000	0.000	35	0.514	0.486	47	0.000	1.000	0.000	0.000	47	0.000	1.000	0.000	40	0.050	0.950	0.000	0.000	47	1.000	0.000
S. alpinus (4)	25	0.000	0.000	1.000	0.000	0.000	25	0.000	1.000	0.000	25	0.000	0.000	1.000	0.000	25	0.000	1.000	24	0.000	0.958	0.042	0.000	25	0.000	1.000	0.000	25	0.000	1.000	0.000	0.000	25	1.000	0.000
S. confluentus (6)	1	0.000	0.000	0.000	1.000	0.000	1	0.000	1.000	0.000	1	1.000	0.000	0.000	0.000	1	1.000	0.000	1	0.000	1.000	0.000	0.000	1	1.000	0.000	0.000	1	0.000	1.000	0.000	0.000	1	1.000	0.000
S. confluentus (6)	13	0.000	0.000	0.000	1.000	0.000	13	0.000	1.000	0.000	13	1.000	0.000	0.000	0.000	13	1.000	0.000	13	0.000	1.000	0.000	0.000	13	1.000	0.000	0.000	13	0.000	1.000	0.000	0.000	12	1.000	0.000
S. confluentus (7)	50	0.000	0.000	0.000	1.000	0.000	50	0.000	1.000	0.000	50	1.000	0.000	0.000	0.000	50	1.000	0.000	50	0.000	1.000	0.000	0.000	50	1.000	0.000	0.000	50	0.000	1.000	0.000	0.000	50	1.000	0.000
S. fontinalis (8)	10	0.000	1.000	0.000	0.000	0.000	10	1.000	0.000	0.000	10	1.000	0.000	0.000	0.000	10	1.000	0.000	10	0.000	1.000	0.000	0.000	10	0.000	1.000	0.000	10	0.000	0.000	1.000	0.000	10	0.000	1.000
S. fontinalis (9)	4	0.000	1.000	0.000	0.000	0.000	4	1.000	0.000	0.000	4	1.000	0.000	0.000	0.000	4	1.000	0.000	4	0.000	1.000	0.000	0.000	4	0.000	1.000	0.000	4	0.000	0.000	1.000	0.000	4	0.000	1.000
S. fontinalis (10)	24	0.000	1.000	0.000	0.000	0.000	24	1.000	0.000	0.000	24	1.000	0.000	0.000	0.000	24	1.000	0.000	24	0.000	1.000	0.000	0.000	24	0.000	1.000	0.000	24	0.000	0.000	1.000	0.000	24	0.000	1.000
S. fontinalis (11)	49	0.000	1.000	0.000	0.000	0.000	49	1.000	0.000	0.000	49	1.000	0.000	0.000	0.000	49	1.000	0.000	49	0.000	1.000	0.000	0.000	49	0.000	1.000	0.000	49	0.000	0.000	1.000	0.000	49	0.000	1.000
S. leucomaenis (12)	5	0.000	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	5	1.000	0.000	0.000	0.000	5	1.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000
S. l. pluvius (13)	5	0.000	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	5	1.000	0.000	0.000	0.000	5	1.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000
S. l. pluvius (14)	5	0.000	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	5	1.000	0.000	0.000	0.000	5	1.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000
S. malma (16)	8	0.000	0.000	1.000	0.000	0.000	8	0.000	1.000	0.000	8	1.000	0.000	0.000	0.000	8	0.833	0.167	8	0.000	1.000	0.000	0.000	8	0.167	0.833	0.000	8	0.000	1.000	0.000	0.000	8	1.000	0.000
S. malma (16)	28	0.000	0.000	1.000	0.000	0.000	28	0.000	1.000	0.000	28	1.000	0.000	0.000	0.000	28	0.940	0.060	28	0.000	1.000	0.000	0.000	28	0.960	0.150	0.000	28	0.000	1.000	0.000	0.000	28	1.000	0.000
S. malma (17)	50	0.000	0.000	1.000	0.000	0.000	50	0.000	1.000	0.000	50	1.000	0.000	0.000	0.000	50	0.990	0.010	50	0.000	1.000	0.000	0.000	50	0.860	0.140	0.000	50	0.000	1.000	0.000	0.000	50	1.000	0.000
S. malma (18)	49	0.000	0.000	1.000	0.000	0.000	49	0.000	1.000	0.000	49	1.000	0.000	0.000	0.000	49	0.970	0.030	49	0.000	1.000	0.000	0.000	49	0.930	0.070	0.000	49	0.000	1.000	0.000	0.000	49	1.000	0.000
S. malma (19)	5	0.000	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	0.000	1.000	0.000	5	1.000	0.000	5	0.000	0.700	0.000	0.300	5	0.700	0.300	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000
S. m. miyabe (20)	5	0.000	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	0.000	1.000	0.000	5	1.000	0.000	5	0.000	1.000	0.000	0.000	5	0.800	0.200	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000
S. namayoush (21)	8	0.000	0.000	0.000	0.000	1.000	8	0.000	1.000	0.000	8	0.000	1.000	0.000	0.000	8	1.000	0.000	8	0.000	1.000	0.000	0.000	8	1.000	0.000	0.000	8	0.000	1.000	0.000	0.000	8	1.000	0.000
S. namayoush (22)	20	0.000	0.000	0.000	0.000	1.000	20	0.000	1.000	0.000	20	0.000	1.000	0.000	0.000	15	1.000	0.000	20	0.000	1.000	0.000	0.000	20	1.000	0.000	0.000	20	0.000	1.000	0.000	0.000	18	1.000	0.000
S. namayoush (23)	5	0.000	0.000	0.000	0.000	1.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	1.000	0.000

APPENDIX. *(Continued)*

Locus	IDDH*				mIDHP-1*				mIDHP-2*				sIDHP-1*									sIDHP-2*			LDH-A1*				LDH-A2*					LDH-B1*	
Population	(N)	*100	*150	*12	(N)	*100	*238	*400	(N)	*100	*120	*900	(N)	*100	*116	*169	*77	*66	*43	*80	*109	(N)	*100	*116	(N)	*100	*75	*-100	*	(N)	*100	*88	*89	*	(N)	*100	*146
H. perryi (1)	5	1.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	5	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	5	0.000	1.000	5	0.000	0.000	0.000	1.000	5	0.000	0.000	0.000	1.000	5	1.000	0.000
S. alpinus (2)	12	1.000	0.000	0.000	12	0.000	1.000	0.000	12	0.000	1.000	0.000	12	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	12	0.000	1.000	12	0.000	1.000	0.000	0.000	12	0.000	1.000	0.000	0.000	12	0.000	1.000
S. alpinus (3)	47	1.000	0.000	0.000	47	0.000	1.000	0.000	47	0.000	0.830	0.170	33	0.000	0.000	0.000	0.000	0.000	0.078	0.824	0.000	33	0.000	1.000	47	0.000	1.000	0.000	0.000	47	0.000	1.000	0.000	0.000	47	0.000	1.000
S. alpinus (4)	24	1.000	0.000	0.000	25	0.000	1.000	0.000	25	0.000	1.000	0.000	25	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	25	0.000	1.000	26	0.000	1.000	0.000	0.000	26	0.000	1.000	0.000	0.000	25	0.000	1.000
S. confluentus (5)	1	1.000	0.000	0.000	1	0.000	1.000	0.000	1	0.000	1.000	0.000	1	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1	0.000	1.000	1	0.000	1.000	0.000	0.000	1	0.000	1.000	0.000	0.000	23	0.000	1.000
S. confluentus (6)	12	1.000	0.000	0.000	13	0.000	1.000	0.000	13	0.000	1.000	0.000	12	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	12	0.000	1.000	13	0.000	1.000	0.000	0.000	13	0.000	1.000	0.000	0.000	13	0.000	1.000
S. confluentus (7)	50	1.000	0.000	0.000	50	0.000	0.345	0.655	50	0.000	1.000	0.000	50	0.000	1.000	0.000	0.000	0.000	0.000	0.005	0.000	50	0.000	1.000	50	0.000	1.000	0.000	0.000	50	0.000	1.000	0.000	0.000	50	0.000	1.000
S. fontinalis (8)	9	0.000	1.000	0.000	10	0.000	1.000	0.000	10	0.000	1.000	0.000	5	0.000	0.200	0.300	0.500	0.000	0.000	0.000	0.000	5	0.000	1.000	10	0.000	1.000	0.000	0.000	10	0.000	1.000	0.000	0.000	10	0.300	0.700
S. fontinalis (9)	4	0.000	1.000	0.000	4	0.000	1.000	0.000	4	0.000	1.000	0.000	4	0.000	0.000	0.500	0.500	0.000	0.000	0.000	0.000	4	0.000	1.000	4	0.000	0.000	1.000	0.000	4	0.000	1.000	0.000	0.000	4	0.125	0.875
S. fontinalis (10)	24	0.000	1.000	0.000	24	0.000	1.000	0.000	24	0.000	1.000	0.000	23	0.000	0.000	0.435	0.565	0.000	0.000	0.000	0.000	23	0.000	1.000	24	0.000	0.000	1.000	0.000	24	0.000	1.000	0.000	0.000	23	0.130	0.870
S. fontinalis (11)	49	0.000	1.000	0.000	49	0.000	1.000	0.000	49	0.000	1.000	0.000	49	0.000	0.133	0.102	0.765	0.000	0.000	0.000	0.000	49	0.000	1.000	49	0.000	0.000	1.000	0.000	49	0.000	1.000	0.000	0.000	49	0.459	0.541
S. leucomaenis (12)	5	1.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	5	0.000	1.000	5	0.000	1.000	0.000	0.000	5	0.000	0.000	1.000	0.000	5	0.000	1.000
S. l. pluvius (13)	5	0.900	0.000	0.100	5	0.000	1.000	0.000	5	0.000	1.000	0.000	5	0.000	0.300	0.000	0.000	0.000	0.000	0.000	0.700	5	0.000	1.000	5	0.000	1.000	0.000	0.000	5	0.000	0.000	1.000	0.000	5	0.000	1.000
S. l. pluvius (14)	5	1.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	5	0.000	0.400	0.000	0.000	0.000	0.000	0.000	0.600	5	0.000	1.000	5	0.000	1.000	0.000	0.000	5	0.000	0.000	1.000	0.000	5	0.000	1.000
S. malma (15)	9	1.000	0.000	0.000	9	0.000	1.000	0.000	9	0.000	1.000	0.000	9	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	9	0.000	1.000	9	0.000	1.000	0.000	0.000	9	0.000	1.000	0.000	0.000	9	0.000	1.000
S. malma (16)	26	1.000	0.000	0.000	26	0.000	1.000	0.000	26	0.000	1.000	0.000	26	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	26	0.000	1.000	26	0.000	1.000	0.000	0.000	26	0.000	1.000	0.000	0.000	26	0.000	1.000
S. malma (17)	50	1.000	0.000	0.000	50	0.000	1.000	0.000	50	0.000	1.000	0.000	50	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	50	0.000	1.000	50	0.000	1.000	0.000	0.000	50	0.000	1.000	0.000	0.000	49	0.000	1.000
S. malma (18)	49	1.000	0.000	0.000	49	0.000	1.000	0.000	49	0.000	1.000	0.000	49	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	49	0.000	1.000	49	0.000	1.000	0.000	0.000	49	0.000	1.000	0.000	0.000	49	0.000	1.000
S. malma (19)	5	1.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	5	0.000	1.000	5	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	0.000	1.000
S. m. miyabe (20)	5	1.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	5	0.000	1.000	5	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	0.000	1.000
S. nemayoush (21)	8	1.000	0.000	0.000	8	0.000	1.000	0.000	8	0.000	1.000	0.000	8	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	8	0.000	1.000	8	0.000	1.000	0.000	0.000	8	0.000	1.000	0.000	0.000	8	0.000	1.000
S. nemayoush (22)	18	1.000	0.000	0.000	20	0.000	1.000	0.000	20	0.000	1.000	0.000	18	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	18	0.000	1.000	20	0.000	0.000	1.000	0.000	20	0.000	1.000	0.000	0.000	20	0.000	1.000
S. nemayoush (23)	5	1.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	5	0.000	1.000	5	0.000	0.000	1.000	0.000	5	0.000	1.000	0.000	0.000	5	0.000	1.000

Locus	LDH-B2*				LDH-C*			sMDH-A1*a			sMDH-A2*			sMDH-B1, B2*					MEP-1*						MEP-2*				MEP-3*			
Population	(N)	*100	*26	*72	(N)	*100	*56	(N)	*100	*152	*110	(N)	*100	*162	(N)	*100	*48	*66	*76	(N)	*100	*136	*87	*76	*162	(N)	*100	*162	*75	*135	(N)	*100	*91	*82	*96
H. perryi (1)	5	1.000	0.000	0.000	5	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	5	1.000	0.000	0.000	0.000	5	0.000	0.000	0.000	1.000	0.000	5	0.000	0.000	1.000	0.000	5	0.000	0.000	0.000	1.000
S. alpinus (2)	12	0.000	0.000	1.000	12	1.000	0.000	12	0.000	1.000	0.000	12	0.000	1.000	12	0.000	0.000	0.000	1.000	12	0.000	1.000	0.000	0.000	0.000	12	0.000	1.000	0.000	0.000	12	0.000	1.000	0.000	0.000
S. alpinus (3)	47	0.000	0.000	1.000	47	0.968	0.032	47	0.000	1.000	0.000	47	0.000	1.000	47	0.085	0.000	0.000	0.915	47	0.000	1.000	0.000	0.000	0.000	47	0.000	1.000	0.000	0.000	47	0.000	1.000	0.000	0.000
S. alpinus (4)	24	0.000	0.000	1.000	21	1.000	0.000	24	0.000	1.000	0.000	24	0.000	1.000	25	0.400	0.000	0.000	0.600	25	0.000	1.000	0.000	0.000	0.000	25	0.000	1.000	0.000	0.000	25	0.000	1.000	0.000	0.000
S. confluentus (5)	1	0.000	0.000	1.000	1	1.000	0.000	1	0.000	1.000	0.000	1	0.000	1.000	1	0.000	0.000	0.000	1.000	1	0.000	1.000	0.000	0.000	0.000	1	0.000	1.000	0.000	0.000	1	0.000	1.000	0.000	0.000
S. confluentus (6)	13	0.000	0.000	1.000	13	1.000	0.000	12	0.000	1.000	0.000	12	0.000	1.000	13	0.000	0.000	0.000	1.000	13	0.000	1.000	0.000	0.000	0.000	13	0.000	1.000	0.000	0.000	13	0.000	1.000	0.000	0.000
S. confluentus (7)	50	0.000	0.000	1.000	50	0.990	0.010	50	0.000	1.000	0.000	50	0.000	1.000	50	0.000	0.000	0.000	1.000	50	0.000	1.000	0.000	0.000	0.000	50	0.000	1.000	0.000	0.000	50	0.000	1.000	0.000	0.000
S. fontinalis (8)	10	0.000	1.000	0.000	10	1.000	0.000	10	0.000	0.000	1.000	10	0.000	1.000	10	0.050	0.000	0.000	0.950	10	0.000	0.200	0.800	0.000	0.000	10	0.000	1.000	0.000	0.000	10	0.000	1.000	0.000	0.000
S. fontinalis (9)	4	0.000	1.000	0.000	4	1.000	0.000	4	0.000	0.000	1.000	4	0.000	1.000	4	0.125	0.000	0.000	0.875	4	0.000	0.375	0.625	0.000	0.000	4	0.000	1.000	0.000	0.000	4	0.000	1.000	0.000	0.000
S. fontinalis (10)	23	0.000	1.000	0.000	23	1.000	0.000	24	0.000	0.000	1.000	24	0.000	1.000	24	0.083	0.000	0.000	0.917	24	0.000	0.292	0.708	0.000	0.000	24	0.000	1.000	0.000	0.000	24	0.000	1.000	0.000	0.000
S. fontinalis (11)	49	0.000	1.000	0.000	49	1.000	0.000	49	0.000	0.000	1.000	49	0.000	1.000	49	0.133	0.000	0.000	0.867	49	0.000	0.316	0.681	0.000	0.000	49	0.000	1.000	0.000	0.000	49	0.000	1.000	0.000	0.000
S. leucomaenis (12)	5	0.000	0.000	1.000	5	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	5	0.950	0.000	0.050	0.000	5	0.000	1.000	0.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000
S. l. pluvius (13)	5	0.000	0.000	1.000	5	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	5	1.000	0.000	0.000	0.000	5	0.000	1.000	0.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000
S. l. pluvius (14)	5	0.000	0.000	1.000	5	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	5	1.000	0.000	0.000	0.000	5	0.000	1.000	0.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000
S. malma (15)	9	0.000	0.000	1.000	9	0.917	0.083	9	0.000	1.000	0.000	9	0.000	1.000	9	0.000	0.000	0.000	1.000	9	0.000	1.000	0.000	0.000	0.000	9	0.000	1.000	0.000	0.000	9	0.000	1.000	0.000	0.000
S. malma (16)	26	0.000	0.000	1.000	24	0.750	0.250	26	0.000	1.000	0.000	26	0.000	1.000	26	0.000	0.000	0.000	1.000	26	0.000	1.000	0.000	0.000	0.000	26	0.000	1.000	0.000	0.000	26	0.000	1.000	0.000	0.000
S. malma (17)	50	0.000	0.000	1.000	50	0.730	0.270	50	0.000	1.000	0.000	50	0.000	1.000	50	0.000	0.000	0.000	1.000	50	0.000	1.000	0.000	0.000	0.000	50	0.000	1.000	0.000	0.000	50	0.000	1.000	0.000	0.000
S. malma (18)	49	0.000	0.000	1.000	49	0.440	0.560	49	0.000	1.000	0.000	49	0.000	1.000	49	0.000	0.000	0.000	1.000	49	0.000	1.000	0.000	0.000	0.000	49	0.000	1.000	0.000	0.000	49	0.000	1.000	0.000	0.000
S. malma (19)	5	0.000	0.000	1.000	5	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	5	0.000	0.000	0.000	1.000	5	0.000	1.000	0.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	0.000	0.800	0.400	0.000
S. m. miyabe (20)	5	0.000	0.000	1.000	5	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	5	0.800	0.000	0.000	0.400	5	0.000	1.000	0.000	0.000	0.000	5	0.000	1.000	0.000	0.000	5	0.000	0.300	0.700	0.000
S. nemayoush (21)	8	0.000	0.000	1.000	8	1.000	0.000	8	0.000	1.000	0.000	8	0.000	1.000	8	0.000	1.000	0.000	0.000	8	0.000	0.833	0.000	0.000	0.167	8	0.000	0.000	0.000	1.000	8	0.000	1.000	0.000	0.000
S. nemayoush (22)	20	0.000	0.000	1.000	20	1.000	0.000	20	0.000	1.000	0.000	20	0.000	1.000	18	0.000	1.000	0.000	0.000	20	0.000	0.800	0.000	0.000	0.200	20	0.000	0.000	0.000	1.000	20	0.000	1.000	0.000	0.000
S. nemayoush (23)	5	0.000	0.000	1.000	5	1.000	0.000	5	0.000	1.000	0.000	5	0.000	1.000	5	0.000	1.000	0.000	0.000	5	0.000	1.000	0.000	0.000	0.000	5	0.000	0.000	0.000	1.000	5	0.000	1.000	0.000	0.000

APPENDIX. (*Concluded*)

Locus	*MPI**							*PEP-A1**				*PEPA-2**				*PEP-B1**				*PEPD-1**										*PEPD-2**						
	(N)	*100	*91	*93	*97	*67	*98	(N)	*100	*110	*108	(N)	*100	*108	*74	(N)	*100	*110	*70	(N)	*100	*87	*66	*77	*84	*139	*119	*39	(N)	*100	*87	*77	*122	*139	*119	*128
H. perryi (1)	5	0.000	1.000	0.000	0.000	0.000	0.000	5	0.000	0.000	1.000	5	1.000	0.000	0.000	5	0.000	0.000	1.000	5	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	5	0.000	0.000	0.000	0.000	1.000	0.000	0.000
S. alpinus (2)	11	0.000	1.000	0.000	0.000	0.000	0.000	12	0.000	1.000	0.000	12	1.000	0.000	0.000	12	1.000	0.000	0.000	12	0.000	0.000	0.750	0.000	0.000	0.000	0.000	0.250	12	0.000	0.000	0.000	1.000	0.000	0.000	0.000
S. alpinus (3)	46	0.000	1.000	0.000	0.000	0.000	0.000	47	0.000	1.000	0.000	47	1.000	0.000	0.000	47	1.000	0.000	0.000	47	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	47	0.000	0.000	0.000	1.000	0.000	0.000	0.000
S. alpinus (4)	24	0.000	1.000	0.000	0.000	0.000	0.000	23	0.000	1.000	0.000	24	1.000	0.000	0.000	25	1.000	0.000	0.000	25	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	25	0.000	0.000	0.000	1.000	0.000	0.000	0.000
S. confluentus (5)	1	0.000	0.000	0.000	1.000	0.000	0.000	1	0.000	1.000	0.000	1	0.000	0.000	1.000	1	1.000	0.000	0.000	1	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1	0.000	0.000	0.000	0.000	0.000	0.000	1.000
S. confluentus (6)	13	0.000	0.000	0.000	1.000	0.000	0.000	13	0.000	1.000	0.000	13	0.000	0.000	1.000	13	0.962	0.038	0.000	13	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	13	0.000	0.000	0.000	0.000	0.000	0.000	1.000
S. confluentus (7)	50	0.000	0.000	0.000	1.000	0.000	0.000	50	0.000	1.000	0.000	50	0.000	0.000	1.000	50	1.000	0.000	0.000	50	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	50	0.000	0.000	0.000	0.000	0.000	0.000	1.000
S. fontinalis (8)	10	0.000	0.000	1.000	0.000	0.000	0.000	10	0.000	1.000	0.000	10	1.000	0.000	0.000	10	1.000	0.000	0.000	10	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	10	0.000	0.000	0.000	0.000	0.000	1.000	0.000
S. fontinalis (9)	4	0.000	0.000	1.000	0.000	0.000	0.000	4	0.000	1.000	0.000	4	1.000	0.000	0.000	4	1.000	0.000	0.000	4	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	4	0.000	0.000	0.000	0.000	0.000	1.000	0.000
S. fontinalis (10)	24	0.083	0.000	0.917	0.000	0.000	0.000	24	0.000	1.000	0.000	24	1.000	0.000	0.000	24	1.000	0.000	0.000	24	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	24	0.000	0.000	0.000	0.000	0.000	1.000	0.000
S. fontinalis (11)	49	0.122	0.000	0.878	0.000	0.000	0.000	49	0.000	1.000	0.000	49	1.000	0.000	0.000	49	1.000	0.000	0.000	49	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	49	0.000	0.000	0.000	0.000	0.000	1.000	0.000
S. lausomeanis (12)	5	1.000	0.000	0.000	0.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	0.000	1.000	5	0.000	1.000	0.000	5	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	5	0.000	0.000	0.000	1.000	0.000	0.000	0.000
S. l. pluvius (13)	5	1.000	0.000	0.000	0.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	0.000	1.000	5	0.800	0.400	0.000	5	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	5	0.000	0.000	0.000	1.000	0.000	0.000	0.000
S. l. pluvius (14)	5	1.000	0.000	0.000	0.000	0.000	0.000	5	0.000	1.000	0.000	5	0.000	0.000	1.000	5	0.000	1.000	0.000	5	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	5	0.000	0.000	0.000	1.000	0.000	0.000	0.000
S. malma (15)	6	0.000	1.000	0.000	0.000	0.000	0.000	6	0.000	1.000	0.000	6	1.000	0.000	0.000	6	1.000	0.000	0.000	6	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	6	0.000	0.000	0.500	0.500	0.000	0.000	0.000
S. malma (16)	23	0.042	0.938	0.000	0.000	0.021	0.000	26	0.000	1.000	0.000	26	1.000	0.000	0.000	26	0.867	0.000	0.133	26	0.000	0.000	0.000	0.840	0.080	0.000	0.000	0.000	26	0.000	0.000	0.020	0.880	0.040	0.040	0.000
S. malma (17)	48	0.010	0.906	0.000	0.000	0.083	0.000	50	0.000	1.000	0.000	50	1.000	0.000	0.000	49	0.806	0.000	0.194	50	0.000	0.000	0.000	0.920	0.080	0.000	0.000	0.000	50	0.000	0.000	0.000	0.820	0.180	0.000	0.000
S. malma (18)	48	0.000	0.875	0.000	0.000	0.125	0.000	49	0.000	1.000	0.000	48	1.000	0.000	0.000	48	1.000	0.000	0.000	49	0.000	0.000	0.000	0.690	0.310	0.000	0.000	0.000	48	0.000	0.000	0.000	0.990	0.000	0.010	0.000
S. malma (19)	5	0.000	1.000	0.000	0.000	0.000	0.000	5	0.000	1.000	0.000	5	1.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	5	0.000	0.000	0.000	1.000	0.000	0.000	0.000
S. m. miyabe (20)	5	0.500	0.500	0.000	0.000	0.000	0.000	5	0.000	1.000	0.000	5	1.000	0.000	0.000	5	0.700	0.300	0.000	5	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	5	0.000	0.000	0.000	1.000	0.000	0.000	0.000
S. namaycush (21)	6	0.000	0.000	0.000	0.000	0.000	1.000	6	0.000	1.000	0.000	6	0.000	1.000	0.000	6	1.000	0.000	0.000	6	0.000	0.750	0.250	0.000	0.000	0.000	0.000	0.000	6	0.000	1.000	0.000	0.000	0.000	0.000	0.000
S. namaycush (22)	20	0.000	0.000	0.000	0.000	0.000	1.000	20	0.000	1.000	0.000	20	0.000	1.000	0.000	20	1.000	0.000	0.000	20	0.000	0.800	0.200	0.000	0.000	0.000	0.000	0.000	20	0.000	1.000	0.000	0.000	0.000	0.000	0.000
S. namaycush (23)	5	0.000	0.000	0.000	0.000	0.000	1.000	5	0.000	1.000	0.000	5	0.000	1.000	0.000	5	1.000	0.000	0.000	5	0.000	0.800	0.200	0.000	0.000	0.000	0.000	0.000	5	0.000	1.000	0.000	0.000	0.000	0.000	0.000

Locus	*PEP-LT**						*PGDH**			*PGM-1**				*PGM-2**				*PRP-3**			*SOD**						*TPI-1**		
Population	(N)	*100	*88	*126	*69	*66	(N)	*100	*110	(N)	*-100	*-83	*-66	null	(N)	*100	*116	*'	(N)	*100	*110	(N)	*100	*178	*162	*200	*209	(N)	*-100	*-60
H. perryi (1)	5	1.000	0.000	0.000	0.000	0.000	5	0.000	1.000	5	0.000	1.000	0.000	0.000	5	0.000	0.000	1.000	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000	5	0.000	1.000
S. alpinus (2)	12	1.000	0.000	0.000	0.000	0.000	12	0.000	1.000	12	0.000	0.000	1.000	0.000	12	0.000	1.000	0.000	12	0.000	1.000	11	0.000	1.000	0.000	0.000	0.000	12	1.000	0.000
S. alpinus (3)	47	0.000	1.000	0.000	0.000	0.000	47	0.000	1.000	47	0.000	0.000	1.000	0.000	47	1.000	0.000	0.000	47	0.000	1.000	44	0.000	1.000	0.000	0.000	0.000	47	1.000	0.000
S. alpinus (4)	22	1.000	0.000	0.000	0.000	0.000	24	0.000	1.000	25	0.000	0.000	1.000	0.000	25	1.000	0.000	0.000	22	0.000	1.000	25	0.000	1.000	0.000	0.000	0.000	25	1.000	0.000
S. confluentus (5)	1	1.000	0.000	0.000	0.000	0.000	1	0.000	1.000	1	1.000	0.000	0.000	0.000	1	1.000	0.000	0.000	1	0.000	1.000	1	0.000	0.000	0.000	1.000	0.000	1	1.000	0.000
S. confluentus (6)	13	1.000	0.000	0.000	0.000	0.000	12	0.000	1.000	12	1.000	0.000	0.000	0.000	12	1.000	0.000	0.000	13	0.000	1.000	13	0.000	0.000	0.000	1.000	0.000	13	1.000	0.000
S. confluentus (7)	50	1.000	0.000	0.000	0.000	0.000	50	0.000	1.000	50	1.000	0.000	0.000	0.000	50	1.000	0.000	0.000	50	0.000	1.000	50	0.000	0.000	0.000	1.000	0.000	50	1.000	0.000
S. fontinalis (8)	10	0.000	1.000	0.000	0.000	0.000	10	0.000	1.000	10	0.800	0.000	0.000	0.200	10	1.000	0.000	0.000	10	0.000	1.000	10	1.000	0.000	0.000	0.000	0.000	10	0.000	1.000
S. fontinalis (9)	4	0.000	1.000	0.000	0.000	0.000	4	0.000	1.000	4	0.750	0.000	0.000	0.250	4	1.000	0.000	0.000	4	0.000	1.000	4	1.000	0.000	0.000	0.000	0.000	4	0.000	1.000
S. fontinalis (10)	24	0.000	1.000	0.000	0.000	0.000	24	0.000	1.000	24	0.917	0.000	0.000	0.083	24	1.000	0.000	0.000	22	0.000	1.000	24	1.000	0.000	0.000	0.000	0.000	24	0.000	1.000
S. fontinalis (11)	49	0.000	1.000	0.000	0.000	0.000	49	0.000	1.000	49	0.980	0.000	0.000	0.020	49	0.928	0.071	0.000	49	0.000	1.000	49	1.000	0.000	0.000	0.000	0.000	49	0.022	0.978
S. lausomeanis (12)	5	0.800	0.000	0.000	0.200	0.000	5	0.000	1.000	5	1.000	0.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	5	0.000	0.000	0.000	1.000	0.000	5	0.000	1.000
S. l. pluvius (13)	5	1.000	0.000	0.000	0.000	0.000	5	0.000	1.000	5	1.000	0.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	5	0.000	0.000	0.000	1.000	0.000	5	0.000	1.000
S. l. pluvius (14)	5	1.000	0.000	0.000	0.000	0.000	5	0.000	1.000	5	1.000	0.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	5	0.000	0.000	0.000	1.000	0.000	5	0.000	1.000
S. malma (15)	5	0.000	0.000	0.000	0.000	1.000	6	0.000	1.000	6	1.000	0.000	0.000	0.000	6	1.000	0.000	0.000	6	0.000	1.000	4	0.000	1.000	0.000	0.000	0.000	6	1.000	0.000
S. malma (16)	26	0.000	0.000	1.000	0.000	0.000	23	0.000	1.000	26	1.000	0.000	0.000	0.000	26	1.000	0.000	0.000	26	0.000	1.000	26	0.000	0.810	0.190	0.000	0.000	26	1.000	0.000
S. malma (17)	50	0.000	0.000	1.000	0.000	0.000	50	0.000	1.000	50	1.000	0.000	0.000	0.000	50	1.000	0.000	0.000	50	0.000	1.000	50	0.000	0.730	0.270	0.000	0.000	50	1.000	0.000
S. malma (18)	48	0.000	0.000	1.000	0.000	0.000	48	0.000	1.000	48	1.000	0.000	0.000	0.000	48	0.990	0.010	0.000	50	0.000	1.000	48	0.000	0.550	0.450	0.000	0.000	48	1.000	0.000
S. malma (19)	5	0.000	0.000	0.800	0.000	0.200	5	0.000	1.000	5	1.000	0.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	5	0.000	0.000	0.000	1.000	0.000	5	1.000	0.000
S. m. miyabe (20)	5	0.300	0.000	0.100	0.000	0.600	5	0.000	1.000	5	1.000	0.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	5	0.000	0.000	0.000	1.000	0.000	5	1.000	0.000
S. namaycush (21)	6	1.000	0.000	0.000	0.000	0.000	6	0.000	1.000	6	1.000	0.000	0.000	0.000	6	1.000	0.000	0.000	6	0.000	1.000	6	0.000	0.000	0.000	0.000	1.000	6	1.000	0.000
S. namaycush (22)	20	1.000	0.000	0.000	0.000	0.000	16	0.000	1.000	20	0.900	0.100	0.000	0.000	20	1.000	0.000	0.000	20	0.000	1.000	20	0.000	0.000	0.000	0.000	1.000	20	1.000	0.000
S. namaycush (23)	5	1.000	0.000	0.000	0.000	0.000	5	0.000	1.000	5	1.000	0.000	0.000	0.000	5	1.000	0.000	0.000	5	0.000	1.000	5	0.000	0.000	0.000	0.000	1.000	5	1.000	0.000

[a]Populations 3, 4, 15, 16, and 17 were variable at an *sMDH-A* locus. Though the variant allele had a similar mobility as *S. fontinalis sMDH-A1*110*, it was pooled because of the inability to determine which locus was varying, and also because of the difficulty of scoring heterozygote versus homozygote alternate phenotypes.

Systematics of the Salmonid Genus *Salvelinus* Inferred from Ribosomal DNA Sequences

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DNA sequences of the second internal transcribed spacer (ITS2) of the nuclear ribosomal DNA were determined for six species of the genus *Salvelinus*: *S. alpinus* (Arctic char), *S. malma* (Dolly Varden), *S. confluentus* (bull trout), *S. leucomaenis* (Japanese char), *S. fontinalis* (brook trout), and *S. namaycush* (lake trout), and for *Hucho perryi* (huchen). The ITS2 sequence data (approximately 375 base pairs (bp)) were combined with previously determined sequence data for the ITS1 (approximately 575 bp), giving a total of 981 bp of aligned sequence for each species. Phylogenetic analysis of the aligned sequences was done using maximum parsimony, maximum likelihood, and distance matrix methods with *H. perryi* as an outgroup. The results were consistent with previous work based on comparisons of morphologies, allozymes, and karyotypes. Comparison of these results with those based on mitochondrial DNA sequences suggests that hybridization may have occurred between *S. confluentus* and *S. alpinus* or *S. malma*.

On a déterminé les séquences d'ADN du deuxième espaceur interne transcrit (ITS2) de l'ADN ribosomique chez six espèces du genre *Salvelinus*, soit *S. alpinus* (omble chevalier), *S. malma* (Dolly Varden), *S. confluentus* (omble à tête plate), *S. leucomaenis* (omble japonais), *S. fontinalis* (omble de fontaine) et *S. namaycush* (touladi), ainsi que chez *Hucho perryi* (huchon japonais). Les données sur les séquences d'ITS2 (environ 375 paires de bases) ont été combinées à des données semblables déjà obtenues pour ITS1 (environ 575 paires de bases), soit un total de 981 paires de bases en séquences alignées pour chaque espèce étudiée. On a ensuite effectué une analyse phylogénétique de ces séquences (parsimonie maximum, maximum de vraisemblance et matrice de distance), *H. perryi* étant considéré comme un groupe isolé. Les résultats concordaient avec ceux de travaux antérieurs basés sur des comparaisons de la morphologie, des allozymes et des caryotypes. En outre, une comparaison des résultats obtenus dans le cadre de la présente étude et les résultats de l'étude des séquences d'ADN mitochondrial porte à croire qu'il y a peut-être eu hybridation entre *S. confluentus* et *S. alpinus* ou *S. malma*.

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Phylogenetic relationships among the fishes of the genus *Salvelinus* have been studied by comparing morphologies (Norden 1961; Behnke 1980, 1984, 1989; Cavender 1980; Stearley 1990), development (Balon 1980; Kendall and Behnke 1984), allozymes (Johnson 1984; Crane 1991), karyotypes (Cavender 1984; Cavender and Kimura 1989; Hartley 1987; Phillips et al. 1989a), and mitochondrial DNA restriction sites (Gyllensten and Wilson 1987, Grewe et al. 1990). In spite of considerable effort, major problems remain in resolving the systematics of this genus. Recently, new information was obtained from restriction maps of ribosomal DNA (Phillips et al. 1992) and from a comparison of the sequences of the first internal transcribed spacer (ITS1) of the ribosomal DNA (Pleyte et al. 1992). The internal transcribed spacers of the ribosomal DNA (ITS1 and ITS2) are especially suitable for phylogenetic comparisons among closely related species because they represent more rapidly evolving regions than those coding for the rRNAs (reviewed in Gerbi 1985). The ITS1 was used successfully in a phylogenetic analysis of the higher primates (Gonzales et al. 1990). The alternation of highly conserved coding regions with less conserved spacer regions makes it possible to utilize the polymerase chain reaction (PCR) technique (Saiki et al. 1988) to rapidly amplify specific regions for direct sequencing. In this paper, we report the ITS2 sequences of the ribosomal DNA from six species of the genus *Salvelinus* and the results of a combined phylogenetic analysis of approximately 1000 bp of aligned sequences from the two internal transcribed spacers using *Hucho perryi* as an outgroup.

Materials and Methods

Tissue samples for DNA extraction were obtained from the following sources: *S. leucomaenis* (Japanese char) and *H. perryi* (huchen) from the Hokkaido Fish Hatchery, Hokkaido, Japan; *S. namaycush* (lake trout) Marquette stock from Iron River Fish Hatchery, Iron River, Wisconsin; *S. fontinalis* (brook trout) from Wisconsin Department of Natural Resources, St. Croix, Wisconsin; *S. confluentus* (bull trout) from Arrow Lake, British Columbia; *S. alpinus* (Arctic char) from Nauyuk Lake, Northwest Territories; and *S. malma* (Dolly Varden) from Auke Creek, Alaska. DNA was isolated from liver by phenol extraction of proteins (Sambrook et al. 1989).

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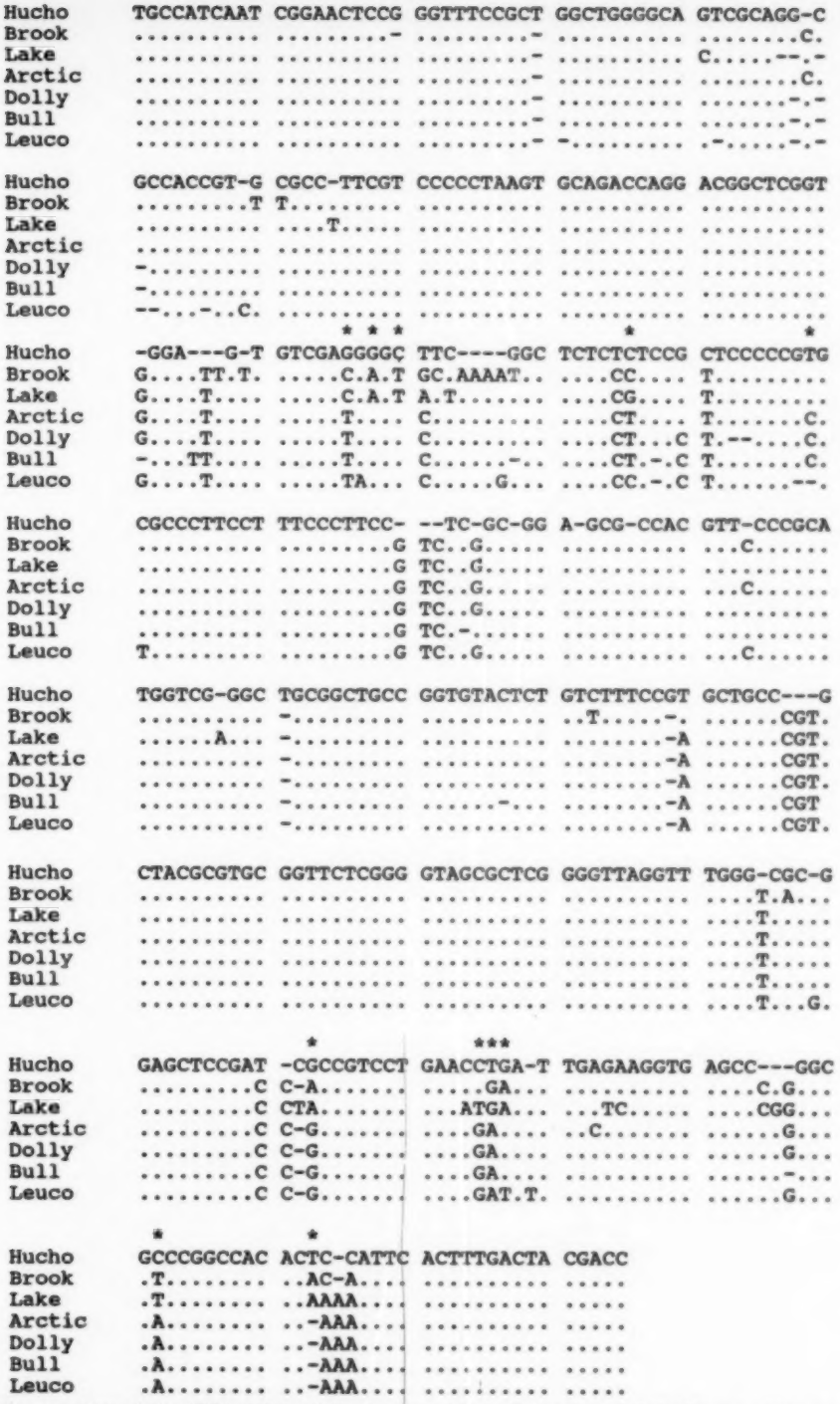
Hucho TGCCATCAAT CGGAACTCCG GGTTTCCGCT GGCTGGGGCA GTCGCAGG-C
Brook ....- .....- ....C.
Lake ....- ..... C.....--.-
Arctic ....- ....C.
Dolly ....- ....-.-
Bull ....- ....-.-
Leuco ....- -..... .-.....-.-
Hucho GCCACCGT-G CGCC-TTCGT CCCCCTAAGT GCAGACCAGG ACGGCTCGGT
Brook .....T T..... ....
Lake ....T..... ....
Arctic ....
Dolly -..... ....
Bull -..... ....
Leuco --...-..C. ....
* * * * *
Hucho -GGA---G-T GTCGAGGGGC TTC----GGC TCTCTCTCCG CTCCCCCGTG
Brook G....TT.T. .....C.A.T GC.AAAAT.. ....CC.... T.....
Lake G....T.... .....C.A.T A.T..... ....CG.... T.....
Arctic G....T.... .....T.... C..... ....CT.... T.....C.
Dolly G....T.... .....T.... C..... ....CT...C T.--....C.
Bull -...TT.... .....T.... C.....-.. ....CT.-.C T.....C.
Leuco G....T.... .....TA... C.....G... ....CC.-.C T.....--.
Hucho CGCCCTTCCT TTCCCTTCC- --TC-GC-GG A-GCG-CCAC GTT-CCCGCA
Brook .....G TC..G..... ....C.....
Lake .....G TC..G..... ....
Arctic .....G TC..G..... ....C.....
Dolly .....G TC..G..... ....
Bull .....G TC.-..... ....
Leuco T.....G TC..G..... ....C.....
Hucho TGGTCG-GGC TGCGGCTGCC GGTGTACTCT GTCTTTCCGT GCTGCC---G
Brook ..... -..... ....T.....-. .....CGT.
Lake .....A... -..... ....-A .....CGT.
Arctic ..... -..... ....-A .....CGT.
Dolly ..... -..... ....-A .....CGT.
Bull ..... -..... .....-... .....-A .....CGT
Leuco ..... -..... ....-A .....CGT.
Hucho CTACGCGTGC GGTTCTCGGG GTAGCGCTCG GGGTTAGGTT TGGG-CGC-G
Brook ....T.A...
Lake ....T.....
Arctic ....T.....
Dolly ....T.....
Bull ....T.....
Leuco ....T...G.
* *** *
Hucho GAGCTCCGAT -CGCCGTCCT GAACCTGA-T TGAGAAGGTG AGCC---GGC
Brook .....C C-A..... .....GA... ....C.G...
Lake .....C CTA..... ...ATGA... ...TC..... ....CGG...
Arctic .....C C-G..... ....GA.... ..C..... .....G...
Dolly .....C C-G..... ....GA.... ....G...
Bull .....C C-G..... ....GA.... ....-...
Leuco .....C C-G..... ....GAT.T. ....G...
* *
Hucho GCCCGGCCAC ACTC-CATTC ACTTTGACTA CGACC
Brook .T..... ..AC-A.... ....
Lake .T..... ..AAAA.... ....
Arctic .A..... ..-AAA.... ....
Dolly .A..... ..-AAA.... ....
Bull .A..... ..-AAA.... ....
Leuco .A..... ..-AAA.... ....

FIG. 1. The aligned sequences of the ITS2 rDNA of *Hucho perryi* and six species of *Salvelinus*. -, gaps; ., sites in common; *, informative sites.

The ITS2 region of the rDNA was amplified from genomic DNA using PCR. Primers for amplification (forward: 5′CTA CGC CTG TCT GAG TGT C 3′; reverse: 5′ATA TGC TTA AAT TCA GCG GG 3′) were designed from the 28S and 5.8S regions flanking the ITS2 based on published sequences of these regions in *Xenopus laevis* (Salim and Maden 1981),

TABLE 1. Matrix of percent sequense divergences among the ITS2s of the ribosomal DNA for six species of *Salvelinus* and *Hucho perryi* calculated using two options: gaps are missing data or gaps are a fifth base. Absolute distances are shown below the diagonal and mean distances (adjusted for missing data) are shown above the diagonal.

	H. perryi	*S. fontinalis*	*S. namaycush*	*S. alpinus*	*S. malma*	*S. confluentus*	*S. leucomaenis*
				Gaps are missing data			
H. perryi	—	0.089	0.104	0.069	0.070	0.076	0.071
S. fontinalis	31	—	0.050	0.055	0.053	0.060	0.048
S. namaycush	36	18	—	0.052	0.050	0.059	0.042
S. alpinus	24	20	19	—	0.003	0.011	0.017
S. malma	24	19	18	1	—	0.008	0.014
S. confluentus	26	21	21	4	3	—	0.023
S. leucomaenis	24	17	15	6	5	8	—
				Gaps are a fifth base			
H. perryi	—	0.158	0.169	0.132	0.135	0.143	0.158
S. fontinalis	61	—	0.086	0.070	0.091	0.114	0.099
S. namaycush	65	33	—	0.075	0.075	0.101	0.091
S. alpinus	51	27	29	—	0.026	0.052	0.057
S. malma	52	35	29	10	—	0.036	0.047
S. confluentus	55	44	39	20	14	—	0.068
S. leucomaenis	61	38	35	22	18	26	—

and *Oncorhynchus mykiss* (rainbow trout) (Nazar and Roy 1978).

PCR volumes of 100 μL consisted of 1 μg of genomic DNA, 10 μL of 10× buffer (0.5M KCl, 0.01 M Tris-H-Cl, pH 8.0, 0.1% gelatin, 0.015 M $MgCl_2$), and 350 pmol of each primer. Amplifications were carried out in a Coy model 60 thermocycler (Coy Laboratory Products, Inc., Ann Arbor, MI) by 30 repetitions of a three-step cycle consisting of denaturation at 94°C for 1.5 min, annealing at 55°C for 2 min, and extension at 72°C for 3 min, and ending with a final extension at 72°C for 7 min. Products were purified using magic PCR preparation columns (Promega, Madison, WI).

The double-stranded PCR products were sequenced either directly or blunt-end cloned in M13 for production of single-stranded sequencing templates. Several clones were sequenced for each species. The Sanger et al. (1977) dideoxy sequencing method was used with Sequenase (U.S. Biochemical, Cleveland, OH) in both cases. For the direct sequencing, a biotinylated 28S primer was used in the amplification, and magnetic beads (Dynal Inc., Great Neck, NY) were used to separate the strands for sequencing.

The DNA sequences were aligned by eye to yield a minimal number of evolutionary changes, with gaps inserted to maintain the alignment. For the cladistic analysis, gaps were considered to be missing data, except in the case of shared unique insertions or deletions in which the inserted base pairs were different from the adjacent base pairs. Informative sites involving base changes were identified as those for which at least two different base pairs were present in at least two of the species. Unique insertions or deletions as defined above of more than one base pair found in two or more species were also considered to be informative sites.

Pairwise sequence divergences were calculated from the aligned sequences both with and without the gaps using the GAPMODE = missing data and GAPMODE = NEWDATA options of the PAUP (Swofford 1985) program. Genetic distances were determined by the Kimura (1980) two-parameter method using the DNADIST option of the PHYLIP program (Felsenstein 1990). Trees were constructed from the genetic distances with the FITCH option of PHYLIP, which uses the Fitch and Margoliash (1967) method, and with the NEIGHBOR option, which includes the neighbor-joining method (Saitou and Nei 1987) and the UPGMA method (Sokal and Michener 1958).

Cladograms were constructed with *H. perryi* as an outgroup using two computer programs. First, PAUP, the maximum-parsimony program of Swofford (1985), was used with the exhaustive method, and the data was bootstrapped 500 times, retaining branches occurring at least 50% of the time. Second, the maximum likelihood algorithm of PHYLIP was used. The trees produced by the PAUP analysis were input as user trees and compared for significance with the tree obtained with the DNAML option of the PHYLIP program. The PHYLIP DNABOOT program was used to generate a likelihood value for each branch from 500 random resamplings of the data, repeated 20 times.

Results

ITS2 Data

The sequences of the ITS2 regions are shown in Fig. 1. The length of the ITS2 in the aligned sequences was 385 base pairs with an average GC content of 67%. If gaps are counted as missing data (Table 1), the seven species are 89.6% identical in sequence, and the six *Salvelinus* species share 94% identical sites. If gaps are treated as a fifth base, then the seven species are 83.1% identical and the six species share 90.1% identical sites (Table 1). The transition to transversion rate was 1:1, and 62% of the 82 variable sites in the aligned sequences were insertions or deletions. There were 11 informative sites for the cladistic analysis when gaps were treated as missing data (see Fig. 1).

One minimum length tree of 59 steps and two trees of 60 steps were generated by an exhaustive search using the PAUP program. In all of these trees, *S. fontinalis* and *S. namaycush* placed together as sister species on one branch, and the relationships among *S. alpinus*, *S. malma*, and *S. confluentus* were an unresolved trichotomy. The trees differed in the placement of *S. leucomaenis*, which grouped with

TABLE 2. Matrix of percent sequence divergences among the combined data from the ITS1 and ITS2 of the ribosomal DNA for six species of *Salvelinus* and *Hucho perryi* calculated using two options: gaps are missing data or gaps are a fifth base. Absolute distances are shown below the diagonal and mean distances (adjusted for missing data) are shown above the diagonal.

	H. perryi	*S. fontinalis*	*S. namaycush*	*S. alpinus*	*S. malma*	*S. confluentus*	*S. leucomaenis*
				Gaps are missing data			
H. perryi	—	0.089	0.087	0.070	0.072	0.075	0.083
S. fontinalis	80	—	0.051	0.051	0.051	0.064	0.070
S. namaycush	78	47	—	0.050	0.050	0.062	0.068
S. alpinus	63	47	46	—	0.004	0.031	0.045
S. malma	65	47	46	4	—	0.031	0.045
S. confluentus	67	58	56	28	28	—	0.034
S. leucomaenis	74	64	62	41	41	31	—
				Gaps are a fifth base			
H. perryi	—	0.144	0.137	0.119	0.121	0.129	0.148
S. fontinalis	141	—	0.071	0.073	0.082	0.109	0.116
S. namaycush	134	70	—	0.065	0.075	0.098	0.109
S. alpinus	117	72	64	—	0.016	0.063	0.077
S. malma	119	80	74	16	—	0.063	0.080
S. confluentus	127	107	96	62	62	—	0.065
S. leucomaenis	145	114	107	76	78	64	—

S. fontinalis and *S. namaycush* in the minimum length tree (Fig. 2), and either on a branch by itself or with *S. alpinus*, *S. malma*, and *S. confluentus* in the two trees that were one step longer. Bootstrap analysis of the sequences indicated that there was relatively weak support for the placement of *S. leucomaenis* with *S. fontinalis* and *S. namaycush*, because this branch was obtained only 63% of the time. A tree similar to the minimum length tree from the parsimony analysis was generated from a matrix of genetic distance values using the FITCH and NEIGHBOR options of the PHYLIP program. The only difference was that the trichotomy was replaced by one branch with *S. malma* and *S. confluentus* and an adjacent branch with *S. alpinus*.

Combined Analysis of ITS1 and ITS2 Data

Percent sequence divergences for the combined data from the ITS1 and ITS2 are shown in Table 2 for the case when gaps are considered as missing data and for the case when gaps are considered as a fifth base. There were 38 informative sites for the cladistic analysis when gaps were treated as missing data.

Maximum parsimony analysis of the combined data from the ITS1 and ITS2 with the PAUP program generated one minimum length tree of 176 steps, which is shown in Fig. 3. In this tree, there are the same three sister groups that were obtained in the parsimony analysis of the ITS1 data (Pleyte et al. 1992), with the sister group of *S. alpinus* and *S. malma* placing with the sister group of *S. confluentus* and *S. leucomaenis*. In the next shortest tree of 177 steps, the sister group of *S. fontinalis* and *S. namaycush* placed with the sister group of *S. alpinus* and *S. malma*. The third possible arrangement of the three sister groups was found in the next shortest tree of 180 steps. Bootstrap values indicated strong support for the three sister groups, but relatively weak support for the arrangement of these subgroups.

Analysis of a matrix of genetic distances, using both the FITCH and NEIGHBOR options of PHYLIP, gave the same tree as the minimum length tree obtained in the parsimony analysis. This tree was also obtained from the maximum likelihood analysis with the DNAML option of PHYLIP. The USER TREE option was used to compare the three different trees obtained in the parsimony analysis, which vary in the arrangement of the sister groups. Tree 1 is the best, but significance analysis (Kishino and Hasegawa 1989) indicates the other trees are not significantly different.

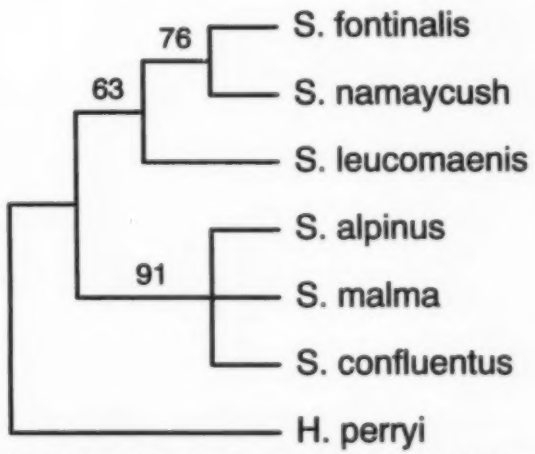

FIG 2. Single minimum length tree generated by the PAUP program with the aligned sequences from the ITS2 of the rDNA for the six species of *Salvelinus* using *Hucho perryi* as an outgroup. Numbers indicate bootstrap values.

Discussion

ITS2 Structure

The aligned regions of the ITS2 of the six species of *Salvelinus* had a length of 385 bp with 94% sequence similarity if gaps are omitted and 90.1% sequence similarity if gaps are included. A comparison of the ITS2 regions of five species of Pacific salmon, *O. tshawytscha* (chinook salmon), *O. kisutch* (coho salmon) *O. keta* (chum salmon)

TABLE 3. Summary of the distribution of the synapomorphies[a] in the ITS1 and ITS2 of the ribosomal DNA for six species of *Salvelinus* and *H. perryi*.

	ITS1	ITS2
S. namaycush – S. fontinalis:	479, 508, 578[b]	34,73,253,266,268,270
S. confluentus – S. leucomaenis	303,310,311,403[b],424,482,487,508[b]	—
S. alpinus – S. malma	116,118,120,313,326,327	—
S. alpinus – S. malma – S. confluentus	408	136,149,327

[a]Using the option that gaps are missing data. Numbers refer to base sites in the ITS1 and ITS2.
[b]Indicates a deletion or insertion starting with that base pair of at least two base pairs.

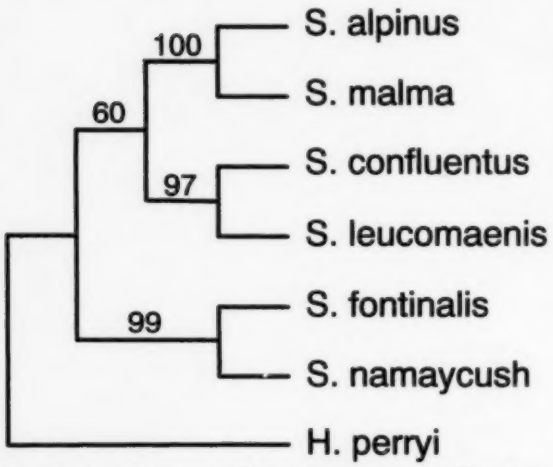

FIG 3. Single minimum length tree generated by the PAUP program with the combined aligned sequences from the ITS1 and ITS2 of the rDNA for the six species of *Salvelinus* using *Hucho perryi* as an outgroup. Numbers indicate bootstrap values.

O. nerka (sockeye salmon), and *O. gorbuscha* (pink salmon) found a size of about 377 bp with 95.1% sequence identity when gaps were omitted (M. Domanico, University of Wisconsin, Milwaukee, WI, personal communication).

Almost no sequence similarity is seen when the ITS2 sequences of the *Salvelinus* species are compared with those of Xenopus (Furlong and Maden 1983), rat (Subrahmanyam et al. 1982), and mouse (Michot et al. 1983). The ITS2 is thought to form a bend allowing the 5.8S to pair with the 28S coding region. Thus, the three-dimensional structure of hairpin loops may be more important than the actual sequence for function.

Systematic Analysis

The exact origin of *Salvelinus* is unknown, but a fossil species, *Salvelinus larsoni*, which has characteristics unique to *Salvelinus* as well as *Hucho*, was found in Idaho from the Miocene period (Stearley and Smith 1983). There is general agreement from morphological data (Smith and Stearley 1989), allozyme data (Crane 1991), and restriction map data from the ribosomal DNA (Phillips et al. 1992) that *Hucho* and *Salvelinus* share a recent common ancestor, so that species in this genus would be the most appropriate outgroups. T. Cavender (Ohio State University, Columbus, OH, personal communication) has reported that a fossil of *Salvelinus* found in western North America is at least 10 million yr old.

Based on morphological characteristics, there appear to be three major subgroups in North America (reviewed in Behnke 1965, 1980). These are *S. namaycush*, confined to northern lakes; *S. fontinalis* found in streams throughout the east and south; and the Arctic char complex, named subgenus *Salvelinus* by Behnke (1980). This group includes *S. alpinus* with a circumpolar distribution in the Arctic; *S. malma*, which occurs sympatrically with *S. alpinus* in the north Pacific; and *S. confluentus* in the Rocky Mountains. In eastern Asia, another distinct species, *S. leucomaenis*, is considered to be more closely related to *S. namaycush* by Savvaitova (1980) and Viktorovsky (1975, 1978), but is placed in the subgenus *Salvelinus* by Behnke. Behnke divides the subgenus *Salvelinus* into the *S. alpinus – S. malma* complex and a group comprising *S. confluentus*, *S. leucomaenis*, and *S. albus* (stone char) from Kamchatka, Russia. Although *S. confluentus* was confused with *S. malma* in the past because they are very similar in external appearence, a detailed osteological analysis by Cavender (1978, 1980) showed that it is a distinct species. Morphological similarities between *S. confluentus* and *S. albus* from Kamchatka have been described by Behnke (1980). *Salvelinus albus*, which was formerly named *S. kronicus* (Glubokowsky and Cheresenev 1981), has a very similar karyotype to *S. confluentus* (reviewed in Cavender 1984; Phillips et al. 1989a).

Examination of karyotypes of all of the species in *Salvelinus* (reviewed in Cavender 1984; Cavender and Kimura 1989; Phillips and Ihssen 1985; Phillips et al. 1989a, 1989b) showed that *S. leucomaenis* from Japan, *S. fontinalis*, and *S. namaycush* have the same karyotype, which is generally agreed to be primitive. Members of the *S. alpinus – S. malma* complex have derived karyotypes that have features not found in the other species. A minimum of four chromosome changes are needed between *S. confluentus* and *S. malma* (Cavender 1984; Phillips et al. 1989a).

Recently, a comprehensive study of allozymes in the genus was completed by Crane (1991) and the data were analyzed using the Fitch–Margoliash algorithm. This study confirmed a close relationship between *S. malma* and *S. alpinus* and between *S. leucomaenis* and *S. confluentus*. Trees based on data from mitochondrial restriction sites (Grewe et al. 1990) and sequencing of a portion of the cytochrome *b* gene (H. McVeigh and W.S. Davidson, Memorial University, St. John's, personal communication) suggested that *S. malma*, *S. alpinus*, and *S. confluentus* were very closely related (1- or 2 bp differences), but that none of them were closely related to the Asian species *S. leucomaenis* (13-bp differences).

In previous work, we sequenced the ITS1 from the six species of *Salvelinus* and *H. perryi*. Phylogenetic analysis of the aligned sequences produced several equally likely topologies. All of these paired *S. confluentus* with *S. leucomaenis* and *S. alpinus* with *S. malma*. In the shortest tree, *S. fontinalis* and *S. namaycush* were also paired. The sequential

branching of these sister pairs was not resolved. The addition of sequences from the ITS2 confirmed the pairing of these three sister groups. Bootstrap analysis of the combined ITS1 and ITS2 sequences gave values of 97–100% for the branches leading to each of the sister groups. The exact branching pattern of these three sister pairs was still not resolved, although the minimum length tree supported Behnke's placement of *S. alpinus*, *S. malma*, *S. confluentus*, and *S. leucomaenis* in the subgenus *Salvelinus*. No support was obtained for the placement of *S. fontinalis* and *S. namaycush* in separate subgenera.

The results obtained from the analysis of the sequences of the ribosomal transcribed spacers differ from those obtained with other molecular data in certain respects. The tree obtained from allozyme data is similar except for the fact that *S. fontinalis* and *S. namaycush* are on separate branches. This is not surprising because this is a distance tree, and our distance data indicates that these species have diverged considerably. The sequence divergence for the ITS2 is 0.05, compared with values of 0.003–0.023 for species in the subgenus *Salvelinus*. One of the strengths of DNA sequence data is that it can be subjected to a parsimony analysis to determine branching relationships. It is important to note that strong support was obtained from both ITS1 and ITS2 sequences for a sister relationship between these two species, and that synapomorphic sites were found to be distributed fairly evenly along both sequences (see Table 3).

The tree obtained from the analysis of the cytochrome *c* gene in the mitochondrial DNA (H. McVeigh and W.S. Davidson, Memorial University, St. John's, personal communication) differs from the trees obtained from allozyme data and the ribosomal DNA sequences in that *S. leucomaenis* is closest to the outgroup of *H. perryi*, with a distance of 13 bp from the other species. The other species in the subgenus *Salvelinus* are all very closely related (differing only by 1 or 2 bp). This result is best explained by hybridization among the North American species in the subgenus *Salvelinus*. If *S. confluentus* was originally more closely related to *S. leucomaenis* but hybridization had occurred between it and species in the *S. alpinus – S. malma* complex, then mitochondrial DNA (mtDNA) from these species could have replaced the original mtDNA. If extensive hybridization had occurred, we would expect a small genetic distance between *S. confluentus* and the species in the *S. alpinus – S. malma* complex. In nuclear genes, we might expect to find heterogeneity with certain regions having a greater similarity to *S. leucomaenis* and other regions having a greater similarity to the other two species. This is in fact the result we obtained: the synapomorphic sites between *S. confluentus* and *S. leucomaenis* are concentrated in the ITS1 region between nucleotides 303 and 508 and none were obtained in the ITS2 (see Table 3).

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Mitochondrial DNA Diversity and Population Structure in Marine Fish Species from the Gulf of Mexico

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Gold, J.R., L.R. Richardson, C. Furman, and F. Sun. 1994. Mitochondrial DNA diversity and population structure in marine fish species from the Gulf of Mexico. Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 205–214.

Variation in mitochondrial DNA (mtDNA) was examined among 693 red drum (*Sciaenops ocellatus*), 300 black drum (*Pogonias cromis*), and 421 red snapper (*Lutjanus campechanus*) sampled from several localities in the Gulf of Mexico. The number of mtDNA genotypes (haplotypes) observed were: 99 in red drum, 37 in black drum, and 68 in red snapper. Variation in mtDNA haplotype frequencies among localities in all three species was not significant, although two mtDNA haplotypes in black drum appeared to be clinally distributed. Maximum-parsimony analysis and phenetic clustering of mtDNA haplotypes and of samples in each species revealed little evidence of phylogeographic structuring. These data indicate that gene flow among localities in each species is sufficient to preclude genetic divergence. Spatial autocorrelation analysis of mtDNA haplotype frequencies revealed an isolation-by-distance effect in red drum and black drum, and indicated that migration between neighboring estuaries or bays in black drum may be less frequent than in red drum. Spatial autocorrelations in red snapper were negative in all distance classes, suggesting little migration even between adjacent localities. Differences in intrapopulational mtDNA diversities were found in all three species, suggesting that geographic differences in effective female population size may occur within each species.

Les variations de l'ADNmt (ADN mitochondrial) on été examinées chez 693 tambours ocellés (*Sciaenops ocellatus*), 300 grands tambours (*Pogonias cromis*) et 421 vivaneaux (*Lutjanus campechanus*) échantillonnés dans plusieurs localités du golfe du Mexique. Le nombre de génotypes d'ADNmt (haplotypes) observé dans chacune des espèces a été le suivant : 99 dans tambours ocellés, 37 dans grands tambours et 68 dans vivaneaux. La variation des fréquences d'haplotypes d'ADNmt entre les localités chez les trois espèces n'était pas statistiquement significative, bien que deux haplotypes observés chez le grand tambour aient semblé présenter une variation clinale. L'analyse du maximum de parcimonie et le regroupement phénétique des haplotypes d'ADNmt et d'échantillons de chacune des espèces n'ont donné que peu d'indices de structure phylogéographique. Ces données indique que le flux génique entre les localités dans chacune des espèces est suffisant pour écarter la divergence génétique. L'analyse d'autocorrélation spatiale des fréquences des haplotypes d'ADNmt a révélé un effet d'isolement par la distance chez le tambour ocellé et le grand tambour, et indiqué que la migration entre les baies ou les estuaires voisins chez le grand tambour peut être moins fréquente que chez le tambour ocellé. Les autocorrélations spatiales chez le vivaneau étaient négatives pour toutes les classes de distance, ce qui indiquerait qu'il y aurait peu de migration entre les diverses localités. Des différences touchant la diversité intrapopulation de l'ADNmt ont été observées dans le cas des trois espèces, ce qui donnerait à entendre qu'il existerait des différences géographiques en ce qui a trait à la taille réelle de la population de femelles dans chacune des espèces.

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Over the past few years, we have carried out studies of spatial and temporal genetic variation among several economically important marine fish species from the northern Gulf of Mexico (Gulf). The central objective of the research has been to determine whether each species is subdivided into discrete subpopulations or stocks. The need for information on stock structure relates to resource allocations and development of fishery regulations on a regional basis. Should separate stocks exist, fishery units could be managed regionally, providing opportunities to adjust regulations to unique needs of subpopulations and resource users. Alternatively, should only a large, single stock exist, management plans could be based on the premise that policies in one region might significantly impact resources in an adjacent region. A second objective of the research has been to determine whether levels of genetic variation differ within the species studied. Information on genetic variation is of interest relative to the concept that levels of genome-wide variation affect probabilities of population survival and fitness (Soulé 1980; Frankel and Soulé 1981).

Most of our studies have employed restriction enzyme site variation in mitochondrial DNA (mtDNA). Briefly, mtDNA in vertebrates is a compact, physically circular, genetically haploid molecule that is uniclonally inherited through the maternal parent (Brown 1983; Avise 1986; Wilson et al. 1985). MtDNA sequence variants are fairly easy to identify, and importantly, do not segregate and recombine during sexual reproduction. This means that effective

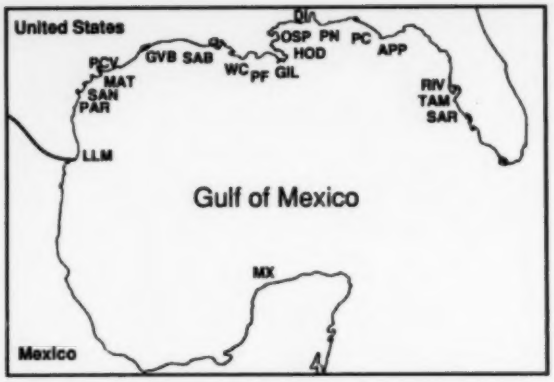

FIG. 1. Localities in the Gulf of Mexico where samples of red drum, black drum, and red snapper were procured. Acronyms are defined in Table 1.

population sizes required to measure genetic impact of population substructuring and gene flow should be at least four times smaller for mtDNA than for nuclear genes (Birky et al. 1983; Templeton 1987). Finally, vertebrate mtDNA appears to have a rapid rate of DNA sequence evolution (Brown 1983; Wilson et al. 1985). The rapid rate of mtDNA sequence change is important relative to identifying discrete subpopulations of recent origin.

In this paper, we summarize studies on geographic variation in mtDNA among three species: red drum (*Sciaenops ocellatus*), black drum (*Pogonias cromis*), and red snapper (*Lutjanus campechanus*). Brief accounts of the biology of the three species, taken primarily from Manooch (1984), Shipp (1986), and Hoese and Moore (1977), are given below. The two drum species are the largest members of the family Sciaenidae and are distributed in coastal and estuarine waters of the western Atlantic from New England through the Gulf to northern Mexico (red drum) or the Yucatan Peninsula (black drum). Both are estuarine dependent as juveniles and move offshore after 1–2 yr (black drum) or 2–3 yr (red drum). Both species are long-lived, although red drum become sexually mature at ages 3–4, whereas black drum become sexually mature at ages 5–6 (Murphy and Taylor 1989; C. Wilson, Coastal Fisheries Institute, Louisiana State University, Baton Rouge, personal communication). Both species form large, offshore schools that can migrate extensively (Matlock 1984, 1987; Swingle et al. 1984; C. Wilson, personal communication). Mass spawning in both species occurs near passes, inlets, river mouths, or bays. Tagging studies in both species have indicated that movement of juveniles among nearshore localities is limited (Matlock and Weaver 1979; Osburn and Matlock 1984; Osburn et al. 1982). No data exist on whether adults return to an area for repeated spawning. Red snapper are the most economically valuable member of the family Lutjanidae and occur in the western Atlantic from North Carolina through the Gulf to the Yucatan Peninsula. Adult red snapper are considered to be sedentary, nonmigratory, and typically associated with offshore, low- and high-relief hard bottoms (Bradley and Bryan 1974; Beaumariage and Bullock 1976). Tagging studies (Fable 1980) indicate that adult movement is limited. Spawning occurs offshore, and eggs and larvae are pelagic. Juveniles are generally found over mud–sand bottoms (Bradley and Bryan 1974; Beaumariage and Bullock 1976).

The primary purposes of this paper are to (1) demonstrate the approaches used to determine whether genetic variation in these species is spatially partitioned, and (2) illustrate that levels and patterns of mtDNA variation differ both within and among species. MtDNA data from red drum are published (Gold and Richardson 1991; Gold et al. 1993), as are part of the mtDNA data from red snapper (Camper et al. 1993).

Materials and Methods

Samples of each species were procured from several localities in the northern Gulf (Fig 1, Table 1) between 1987 and 1991. Tissues were removed from each specimen, frozen in liquid nitrogen, and stored at −80°C. Red drum and black drum were caught in nearshore estuaries and bays using gill and trammel nets, haul seines, and hook and line. Red snapper were caught by hook and line over offshore, hard-bottom substrates. Ages of all but yearling (age zero) red drum (i.e., individuals <300 mm total length) were determined from annuli on otoliths using methods described in Bumguardner (1991). Ages for black drum were not obtained. Otoliths from red snappers are currently under examination by personnel of the Texas Parks and Wildlife Department.

Details regarding assay of individual fish may be found in Gold and Richardson (1991). Our methods employ extraction of genomic DNA with phenol and chloroform, single digestions with (six-base) restriction endonucleases, electrophoresis in 0.8% agarose gels, Southern transfer to nylon membranes, hybridization with mtDNA probes labelled with ^{32}P-dCTP and/or ^{32}P-dATP (3000 Ci/mM) by random priming, and autoradiography. MtDNA fragments are sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII fragment migration distances. Restriction enzymes used for each species were: red drum (*Bam*HI, *Bcl*I, *Eco*RV, *Hin*dIII, *Nco*I, *Nsi*I, *Pst*I, *Pvu*II, *Sca*I, *Spe*I, *Stu*I, *Xba*I, and *Xmn*I); black drum (*Apa*I, *Bgl*II, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Nco*I, *Nhe*I, *Pst*I, *Pvu*II, *Sca*I, *Spe*I, *Ssp*I, *Stu*I, *Xba*I, and *Xmn*I); and red snapper (*Apa*I, *Bcl*I, *Dra*I, *Hin*dIII, *Hpa*I, *Nco*I, *Nhe*I, *Pvu*II, *Sca*I, *Sma*I, *Sst*I, *Stu*I, and *Xba*I). Probes used included a 9.0–9.2 kilobase (kb) fragment of red drum mtDNA inserted into a pTZ-18R plasmid (red drum and black drum) and the entire red snapper mtDNA molecule inserted into bacteriophage λ-DASHII (red snapper). All restriction sites detected in each species have been mapped using single and double digestions (Fig. 2).

Analysis of mtDNA data was facilitated by the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992). Homogeneity of mtDNA haplotype frequencies among geographic samples in each species was tested using (1) log likelihood (G) tests (Sokal and Rohlf 1969), (2) a Monte Carlo randomization (bootstrap) procedure (Roff and Bentzen 1989), and (3) V tests using arcsine, square-root transformed haplotype frequencies (DeSalle et al. 1987). V tests were carried out only on haplotypes found in four or more individuals in each species. Significance levels for multiple tests performed simultaneously were adjusted after Cooper (1968). F_{ST} values (a measure of the variance in mtDNA haplotype frequencies) were calculated using formulae in Weir and Cockerham (1984) and computer programs described in Weir (1990). Estimates of gene flow ($N_e m_f$), the effective

TABLE 1. Locations and number of individuals of three species sampled from the Gulf of Mexico. Acronyms for localities are as used in text and figures.

Locality	Red drum	Black drum	Red snapper†
Mexico			
Merida (MX)	—	—	44
Texas			
Lower Laguna Madre (LLM)	39	52	52
Redfish Bay (PAR)	38	—	60
San Antonio Bay (SAN)	—	53	—
Matagorda Bay (MAT)	—	11	—
Pass Cavallo (PCV)	31	—	—
Galveston Bay (GVB)	68	86	47
Sabine Pass (SAB)	43	—	—
Louisiana			
West Cameron (WC)	—	—	54
Port Fourchon (PF)	—	—	36
Grand Isle (GIL)	90	34	—
Black Bay (HOD)	20	—	—
Mississippi			
Biloxi Bay (OSP)	117	40	—
Alabama			
Dauphin Island (DI)	—	—	53
Florida			
Pensacola (PN)	—	—	25
Panama City (PC)	—	—	50
Apalachicola Bay (APP)	67	13	—
Riviera Bay (RIV)	69	—	—
Tampa Bay (TAM)	—	11	—
Sarasota Bay (SAR)	111	—	—
Totals	693	300	421

†Red snapper were obtained offshore from individual localities

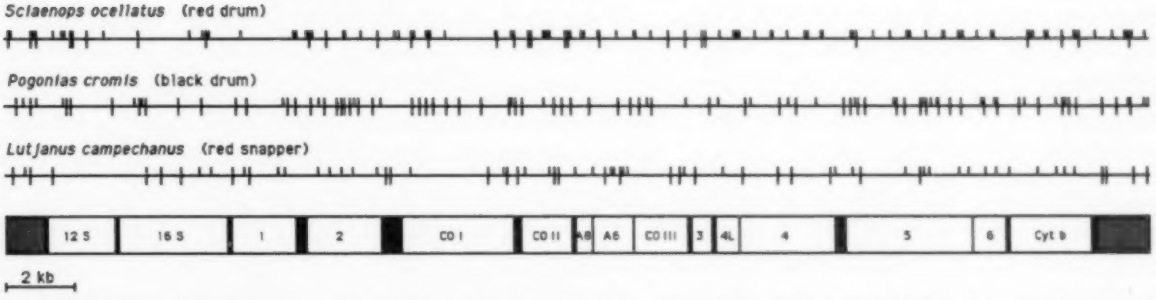

FIG. 2. MtDNA restriction enzyme maps of red drum, black drum, and red snapper. Restriction sites below the line were found in all individuals surveyed; those above the line were polymorphic. Maps are oriented to the human mtDNA gene map. Procedures used to orient maps are given in Schmidt and Gold (1992).

number of female migrants per generation, were calculated using Wright's (1943) island model modified for mtDNA, where $F_{ST} \approx 1/(2N_e m_f + 1)$.

Restriction-site presence–absence (binary) matrices for estimation of nucleotide sequence divergence and for phenetic and parsimony analyses were constructed using individual mtDNA haplotypes and individual sample localities (samples) as operational units. In the latter, restriction sites were scored as present (code 1) if they occurred within a sample, or absent (code 0) if they did not. Nucleotide sequence divergence among mtDNA haplotypes was estimated using restriction sites (Nei and Li 1979); nucleotide sequence divergence among samples (interpopulational divergence) was generated after Nei and Tajima (1981). Phenetic analysis of matrices of nucleotide sequence divergence among haplotypes and among samples was carried out using UPGMA (unweighted pair-group method using arithmetic averages) clustering (Sneath and Sokal 1973). For red drum, year-classes at each locality (sample) were pooled, because homogeneity tests revealed no significant differences between year-classes at any locality. Standard errors for nodes in phenograms were calculated after Nei et al. (1985). Maximum-parsimony analysis of restriction site presence–absence matrices representing all haplotypes and all samples in each species employed MULPARS and CONTREE options in version 3.0s of the Phylogenetic Analysis Using Parsimony (PAUP) program of Swofford (1991). All autapomorphic and symplesiomorphic characters were removed prior to PAUP runs. The strength of nodes produced from maximum-parsimony analysis was assessed by bootstrapping (Felsenstein 1985)

TABLE 2. Summary of mtDNA variation.

Parameter	Red drum	Black drum	Red snapper
Number of individuals	693	300	421
Number of mtDNA restriction sites	104	85	91
Number of mtDNA haplotypes	99	37	68
Nucleon diversity	0.95	0.78	0.75
Nucleotide sequence divergence among haplotypes (%)	0.88	0.48	0.50

TABLE 3. Results of tests for spatial homogeneity in mtDNA haplotype frequencies among localities in the Gulf of Mexico. F_{ST} is a measure of variance in mtDNA haplotype frequencies. $N_e m_f$ is effective number of female migrants per generation.

Test group	No. of localities	No. of haplotypes tested	No. of significant *V* tests	P^b	Results of *G*-tests (*P*)	F_{ST}	$N_e m_f$
Red drum (86)†	11	24	1[a]	0.032	>0.05	−0.002	>10
Red drum (87)†	10	20	1[a]	0.408	≈0.01	0.008	>10
Black drum	8	9	3[a]	0.161	≈0.02	0.011	>10
Red snapper (90)†	3	3	0	0.851	>0.05	0.000	>10
Red snapper (91)†	7	10	0	0.224	>0.05	−0.001	>10

†Red drum from the 1986 and 1987 year-classes (determined from annuli on otoliths); red snapper sampled in 1990 and 1991.
[a]Nonsignificant when corrected for multiple tests.
[b]Probability based on 1000 bootstrap replications (after Roff and Bentzen 1989).

using two different random-number seeds and up to 500 replacements. Minimum-length parsimony networks of mtDNA haplotypes were constructed by connecting composite haplotypes in increments of single site gains or losses.

Spatial autocorrelation analysis of frequencies of common mtDNA haplotypes were used in each species to determine whether haplotype frequencies at any sample locality were independent of haplotype frequencies at neighboring sample localities. The analysis was carried out using the Spatial Autocorrelation Analysis Program (SAAP) of Wartenberg (1989), and involved computation of autocorrelation coefficients (Moran's *I* values) as a function of geographic distance between pairs of localities. Correlograms were used to summarize patterns of geographic variation exhibited by the response surface (geographic distance) of any given variable (mtDNA haplotype frequencies). To minimize "noise" generated by low frequency haplotypes, autocorrelation coefficients were generated from reduced data sets: in red and black drum, only haplotypes found in 10 or more individuals were used (17 haplotypes in red drum and five haplotypes in black drum); in red snapper, only haplotypes found in more than six individuals were used (10 haplotypes). Two SAAP runs were carried out for each species: the first employed equal numbers of pairwise comparisons in each distance class; the second employed equal geographic distances between distance classes. Distance classes were generated by SAAP from input latitude and longitude of each sample locality.

Nucleon diversities (the probability that any two individuals drawn at random will differ in mtDNA haplotype) were calculated after Nei and Tajima (1981) and were based on the total number of mtDNA haplotypes identified. Intrapopulational nucleotide sequence diversities (the average nucleotide difference between any two individuals drawn at random) were estimated after Nei and Tajima (1981) using restriction sites (Nei and Li 1979) for all sample localities in each species.

Results

On average, red drum are the most variable in mtDNA haplotype, and individual mtDNA haplotypes in red drum are more different from one another than are individual haplotypes in black drum and red snapper (Table 2). Based on the conventional rate of evolution for vertebrate mtDNA of 0.01 substitutions·bp^{-1}·lineage^{-1}·1 000 000 yr^{-1} (Brown et al. 1979; Wilson et al. 1985), average divergence times among mtDNA haplotypes are approximately 450 000 yr (red drum) and 250 000 yr (black drum and red snapper).

Following correction for multiple tests, no significant *V* tests for spatial heterogeneity in mtDNA haplotype frequencies were found in any of the three species (Table 3). Prior to correction for multiple tests, two haplotypes (one in each year-class) differed significantly in frequency among samples of red drum, and three haplotypes differed significantly in frequency among samples of black drum. No consistent geographic trends (e.g., clines) in frequency were apparent for the two red drum haplotypes, suggesting that heterogeneity was due simply to random frequency variations at different localities. Two of the three black

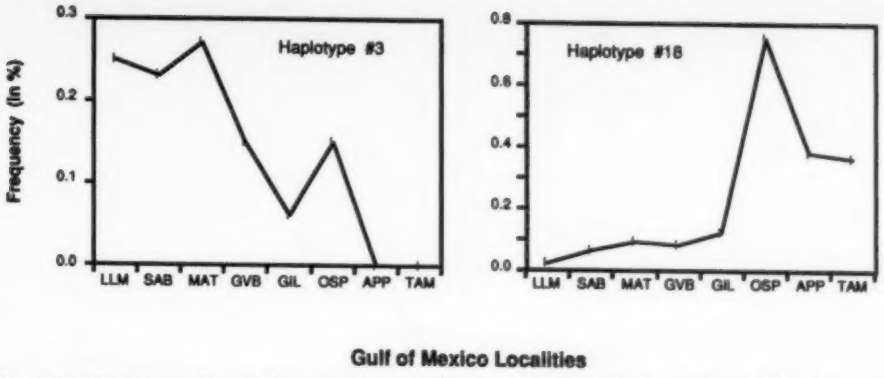

FIG. 3. Frequencies (%) of haplotypes 3 and 18 among black drum at eight localities in the Gulf of Mexico. Acronyms are defined in Table 1.

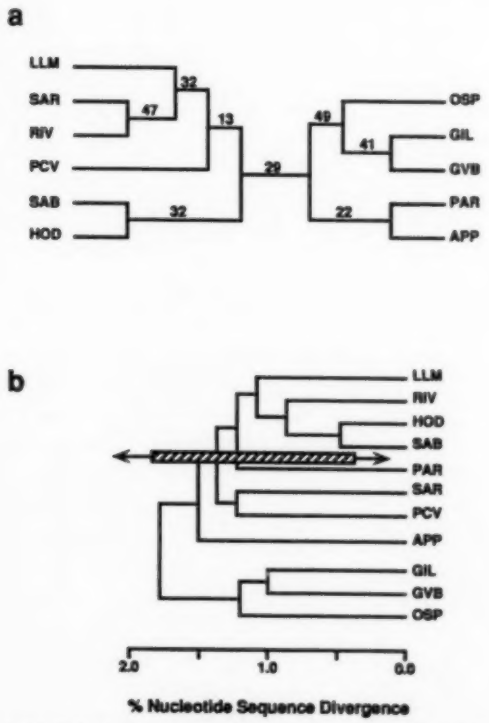

FIG. 4. (a) Strict (unrooted) consensus tree produced by maximum-parsimony analysis of presence–absence restriction-site matrix of red drum samples. Numbers along branches indicate proportion of times a branch (clade) was distinguished in bootstrap analysis. Branch lengths do not represent the number of character state changes. (b) UPGMA cluster analysis of (uncorrected) nucleotide sequence divergence (in percent) among samples of red drum. Hatched bar is standard error of the node it overlies. Acronyms are defined in Table 1.

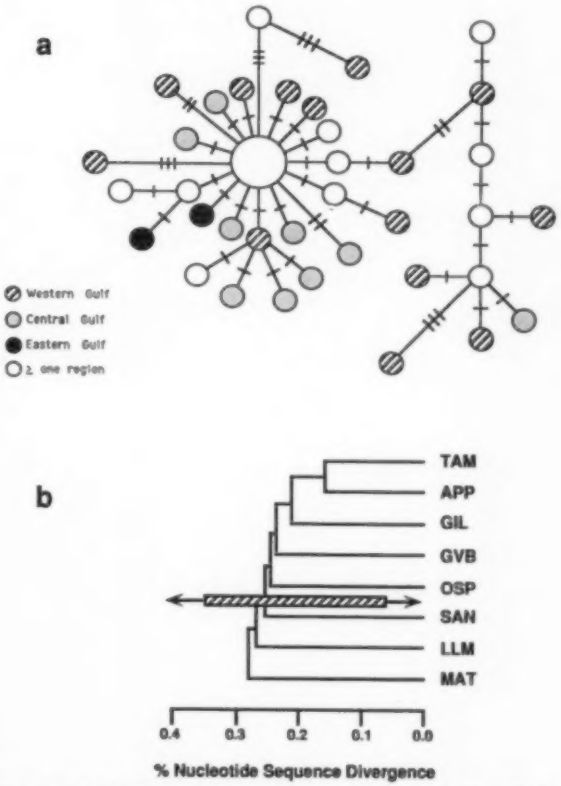

FIG. 5. (a) Minimum-length parsimony network of 37 mtDNA haplotypes in black drum. Branches connecting haplotypes are proportional in length to restriction site changes (hatch marks) required to connect adjacent haplotypes. Western Gulf includes samples from Texas; central Gulf includes samples from Louisiana and Mississippi; eastern Gulf includes samples from Florida (Table 1). (b) UPGMA cluster analysis of (uncorrected) nucleotide sequence divergence (in percent) among samples of black drum. Hatched bar is standard error of the node it overlies. Acronyms are defined in Table 1.

drum haplotypes, however, were clinally distributed: frequencies of haplotype No. 3 were high in the eastern Gulf and low in the western Gulf, whereas frequencies of haplotype No. 18 showed the reverse pattern (Fig. 3). Bootstrap probabilities (after Roff and Bentzen 1989) indicated significant heterogeneity among samples from the 1986 year-class of red drum, and significant G tests were found among samples from the 1987 year-class of red drum and among samples of black drum (Table 3). In no case was a significant

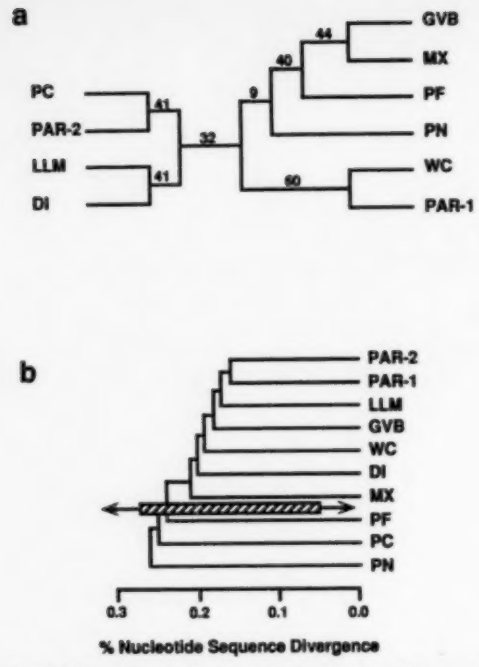

FIG. 6. (a) Strict (unrooted) consensus tree produced by maximum-parsimony analysis of presence–absence restriction-site matrix of red snapper samples. Details are the same as in Fig. 4. (b) UPGMA cluster analysis of (uncorrected) nucleotide sequence divergence (in percent) among samples of red snapper. Hatched bar is standard error of the node it overlies. Acronyms are defined in Table 1. PAR-1 and PAR-2 refer to samples from Port Aransas, Texas, collected in 1990 and 1991, respectively.

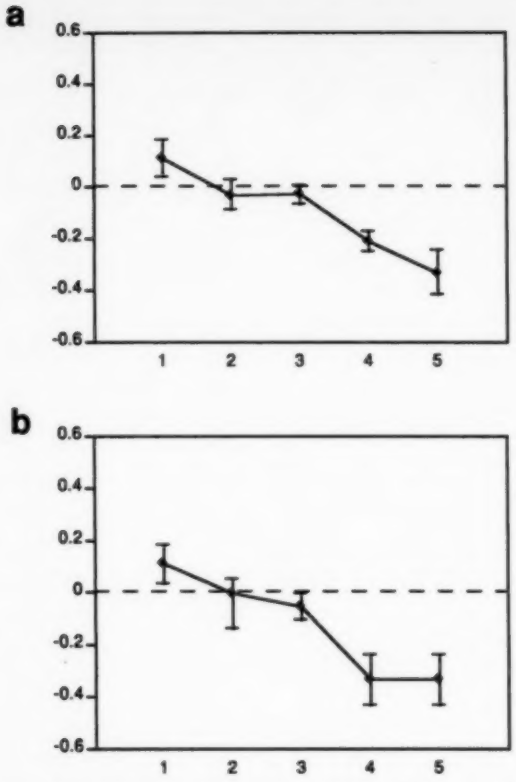

FIG. 7. Correlograms based on spatial autocorrelation analysis of mtDNA haplotype frequencies in red drum. Abscissas: distance classes 1–5 (left to right). Ordinates: mean autocorrelation coefficients (Moran's I values) for each distance class. Bars about each mean value represent one standard error on either side of a mean. (a) Equal frequencies–distance class. (b) equal distances between distance classes.

bootstrap probability and significant *G*-test value obtained for the same test group.

Estimates of F_{ST} ranged from near zero among samples of red drum from the 1986 year-class and red snapper sampled in both 1990 and 1991 to 0.011 among samples of black drum (Table 3). Estimates of $N_e m_f$ (the effective number of female migrants per generation) were greater than 10 in all test groups (Table 3). The estimates of F_{ST} and $N_e m_f$ indicate little genetic subdivision and occurrence of significant gene flow among all test groups.

The strict consensus "gene tree" and phenogram produced from red drum haplotype matrices revealed no evidence of phylogeographic structuring or spatial cohesion of haplotypes. The strict consensus tree produced from the matrix of samples (Fig. 4a) indicated little support for phyletic cohesion of geographically proximate samples. In the UPGMA-derived phenogram of samples (Fig. 4b), standard errors of most nodes were greater than the distance between the first and last nodes, effectively collapsing all nodes and indicating that red drum samples are not strongly differentiated genetically.

One of many possible minimum-length parsimony networks of 37 haplotypes found in black drum (Fig. 5a) required 47 steps. All clades or clusters revealed by maximum-parsimony analysis or UPGMA clustering of haplotype restriction site matrices were linked in the minimum-length parsimony network. Individual haplotypes in the network are identified by occurrence in the western Gulf (samples from Texas), central Gulf (samples from Louisiana and Mississippi), or eastern Gulf (samples from Florida). No clades or clusters of black drum haplotypes occur solely in any of the three regions (i.e., western, central, or eastern Gulf), indicating little evidence of phylogeographic structuring of haplotypes. In the UPGMA-derived phenogram of samples (Fig. 5b), standard errors of most nodes were greater than the distance between the first and last nodes, effectively collapsing all nodes and indicating that black drum samples are not strongly differentiated genetically.

The strict consensus gene tree and phenogram produced from red snapper haplotype matrices revealed no evidence of phylogeographic structuring or cohesion of haplotypes. The strict consensus tree produced from the restriction site matrix of samples (Fig. 6a) indicated little support for phyletic cohesion of geographically proximate samples. In the UPGMA-derived phenogram of samples (Fig. 6b), standard errors of most nodes were greater than the distance between the first and last nodes, effectively collapsing all nodes and indicating that red snapper samples are not strongly differentiated genetically.

In red drum, mean Moran's *I* values were positive in the first distance class, near zero in the second and third distance

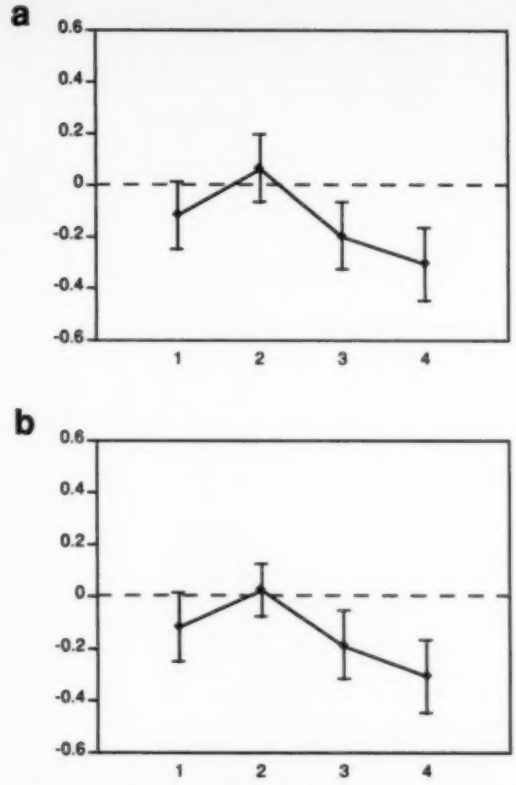

FIG. 8. Correlograms based on spatial autocorrelation analysis of mtDNA haplotype frequencies in black drum. Details are the same as in Fig. 7.

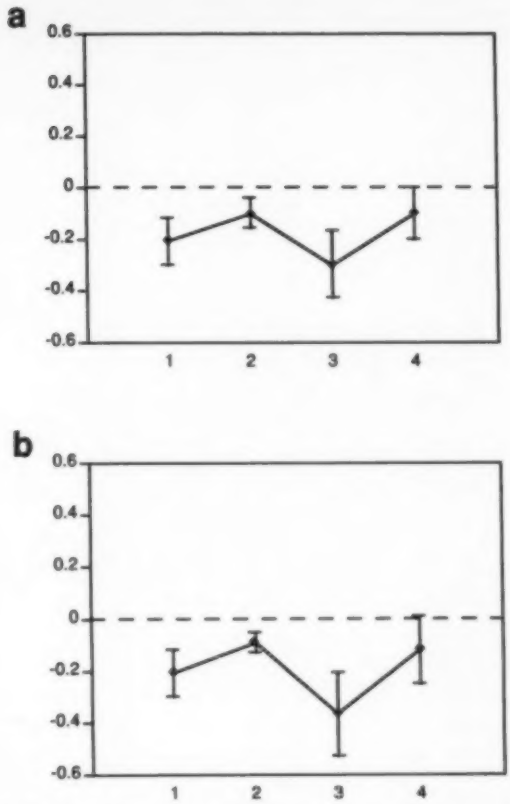

FIG. 9. Correlograms based on spatial autocorrelation analysis of mtDNA haplotype frequencies in red snapper. Details are the same as in Fig. 7.

classes, and negative in the last two distance classes in SAAP runs using equal frequencies or equal distances (Fig. 7). These results indicate autocorrelation among haplotype frequencies as a function of distance between pairs of localities, i.e., red drum in geographically proximate or neighboring localities are more similar genetically than are red drum in more geographically distant localities. In black drum, mean Moran's *I* values were effectively zero in the first two distance classes and negative in the last two distance classes in both SAAP runs (Fig. 8). These results also indicate autocorrelation among haplotype frequencies as a function of distance between pairs of localities, and that black drum in geographically proximate localities are more similar genetically than are black drum in more geographically distant localities. The lower autocorrelation coefficients in the first two distance classes in black drum, as compared with red drum, may indicate that black drum in neighboring localities are less similar genetically than are red drum in neighboring localities. In red snapper, mean Moran's *I* values were negative in all distance classes, with highest negative values occurring in penultimate distance classes in both SAAP runs (Fig. 9). We interpret this somewhat novel pattern as indicating that red snapper in geographically proximate localities are no more similar than are red snapper in geographically distant localities.

Intrapopulational mtDNA diversities appear to vary geographically in all three species (Fig. 10). MtDNA diversity values in both red drum and black drum appear higher in samples from the western Gulf, whereas the reverse appears to occur in red snapper (i.e., mtDNA diversity values appear higher in the eastern Gulf). Based on theoretical studies by Avise et al. (1988), and assuming that generation time and mtDNA mutation rate is the same across samples within each species, differences in intrapopulational mtDNA diversities may reflect differences in effective number of females that gave rise to different samples.

Discussion

Previous population-genetic studies in a variety of commercially and/or recreationally important marine fish species generally have demonstrated homogeneity in nuclear-gene or mtDNA allele frequencies among geographic samples of the same species. Species studied have included milkfish (*Chanos chanos*; Winans 1980), walleye pollock (*Theragra chalcogramma*; Grant and Utter 1980), skipjack tuna (*Katsuwonus pelamis*; Graves et al. 1984), Pacific (*Clupea pallasi*) and Atlantic herring (*Clupea harengus*; Grant and Utter 1984; Kornfield and Bogdanowicz 1987), halibut (*Hippoglossus* spp.; Grant et al. 1984), Atlantic cod (*Gadus morhua*; Mork et al. 1985), Pacific ocean perch (*Sebastes alutus*; Seeb and Gunderson 1988), and bluefish (*Pomatomus saltatrix*; Graves et al. 1992). Observed genetic homogeneity

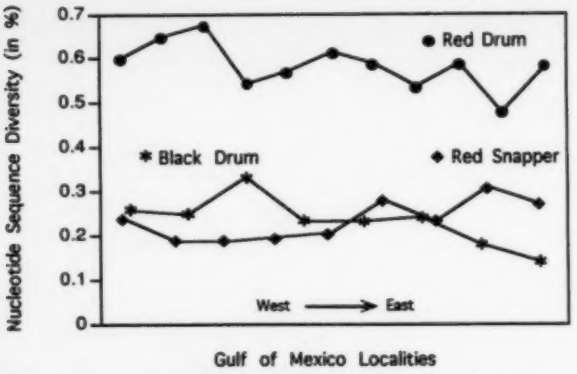

FIG. 10. Geographic variation in intrapopulational mtDNA diversity among samples of red drum, black drum, and red snapper from the Gulf of Mexico. The abscissa is the geographic samples oriented west to east (left to right) in the northern Gulf and the ordinate is the mean intrapopulational mtDNA diversities.

has been considered consistent with the hypothesis that gene flow (migration) among localities is sufficient to preclude significant genetic divergence.

As noted by Camper et al. (1993), there are caveats to the above hypothesis. First, genetic homogeneity does not indisputably establish the existence of a single, panmictic population. Proving a null hypothesis is impossible, and genetic homogeneity is simply consistent with the hypothesis that samples are drawn from a population with the same parametric allele frequencies. Moreover, small amounts of gene flow (e.g., 5%) will homogenize populations genetically, even though a species may be fragmented demographically (D. Campton, Department of Fisheries and Aquaculture, University of Florida, Gainesville, personal communication). A second caveat is that observed homogeneity may reflect historical rather than present-day events. Species could be isolated spatially in the present day, but have had sufficient genetic contact in the recent past to remain indistinguishable in allele frequencies. This possibility is also difficult to test.

The traditional approach of testing homogeneity in allele (mtDNA haplotype) frequencies revealed little evidence of significant genetic differentiation among samples of red drum, black drum, and red snapper from the Gulf. Clinal variation of two haplotypes in black drum, however, suggested the possibility of low-level divergence in this species between the eastern and western Gulf. This hypothesis could be tested further by sampling additional individuals.

In several recent publications, Avise (1989, 1992) and colleagues (Avise et al. 1987; Bowen and Avise 1990) have used a phylogenetic approach to ask whether genealogical lineages are nonrandomly distributed across geographic space. This "phylogeographic" approach has an advantage over traditional frequency-based approaches in that low frequency alleles (mtDNA haplotypes) can contribute to considerations of geographic subdivision. Phylogeographic analysis (including parsimony and phenetic methods) of mtDNA haplotypes and of samples in red drum, black drum, and red snapper, however, also revealed little evidence of geographic subdivision within the Gulf.

In our initial studies (Gold and Richardson 1991; Camper et al. 1993; C. Furman and J.R. Gold, unpublished data), genetic homogeneity and absence of phylogeographic structuring among geographic samples was taken as support for the hypothesis that each of the three species was comprised of a single genetic stock within the Gulf. The data also supported the hypothesis that gene flow throughout the Gulf was sufficient to homogenize haplotype frequencies. For red drum, these hypotheses were compatible with known life history. Both larval and juvenile red drum are largely estuarine dependent, but adults move offshore and form large schools that can migrate extensively (Matlock 1984, 1987, Swingle et al. 1984). For black drum and red snapper, however, life history considerations indicate that migration in the Gulf may be more limited than in red drum. Black drum also are estuarine dependent as larvae and juveniles, but are generally viewed as more sedentary as adults than are red drum (Osburn and Matlock 1984; Cody et al. 1985). For red snapper, the prevailing view is that both adults and juveniles are nonmigratory. This view is based largely on tagging studies and observations that juveniles and adults often exhibit substrate specificity (Bradley and Bryan 1974; Beaumariage and Bullock 1976; Fable 1980).

Different patterns of spatial autocorrelation of mtDNA haplotype frequencies were found in the three species. In red drum, positive autocorrelations were found in geographically proximate or neighboring sample localities, whereas increasingly negative autocorrelations were found with increasing geographic distance between sample localities. This pattern is consistent with an "isolation-by-distance" model where migration of individuals is inversely related to geographic distance (Sokal and Oden 1978). In red drum, migration would be hypothesized to be inversely proportional to distance from the estuary or bay of natal origin. This hypothesis suggests that red drum may be semiisolated spatially, but that overall gene flow within the Gulf is sufficient to maintain similar haplotype frequencies and neutralize potential phylogeographic structuring.

In black drum, the overall pattern of spatial autocorrelation was similar to that in red drum: increasingly negative autocorrelation was found with increasing geographic distance between sample localities. Unlike red drum, however, autocorrelation between geographically proximate sample localities was essentially zero in black drum. We interpret this pattern to indicate that migration of black drum also is inversely related to geographic distance from the estuary or bay of natal origin, but that the frequency of migration between neighboring estuaries or bays in considerably less than that in red drum. This interpretation is consistent with the prevailing view that black drum are less migratory than are red drum.

In red snapper, spatial autocorrelations were either negative or effectively zero, and a pattern of increasingly negative autocorrelation with increasing geographic distance between sample localities was not observed. The negative autocorrelation found between geographically proximate sample localities indicates either (1) little or no effective migration between localities, or (2) environmental heterogeneity and strong "patch" selection where patch size is less than interlocality distances (Sokal and Oden 1978). We favor the first alternative, in part because we assume the mtDNA variants under study are selectively neutral, and in part because little to no migration between contiguous localities also would account for the absence of increasingly negative autocorrelation as distance between localities increases.

The foregoing indicates that spatial subdivision and reduced gene flow among Gulf localities occurs in all three species despite the absence of significant frequency differences and phylogeographic structuring of mtDNA haplotypes. Similar results were found recently among haddock from the western North Atlantic (Zwanenburg et al. 1992), where the existence of reduced gene flow and discrete stocks was inferred from correlations between genetic dissimilarity and geographic distance. Relative to the Gulf species studied here, we hypothesize that gene flow is highest in red drum and lowest in red snapper. This hypothesis is consistent with known life history information in all three species (Matlock 1984, 1987; Bradley and Bryan, 1974; Beaumariage and Bullock, 1976; Osburn and Matlock 1984; Murphy and Taylor 1989; C. Wilson, personal communication).

The variation in intrapopulational mtDNA diversity among samples of all three species is also suggestive of geographic differences within the Gulf. Avise et al. (1988) demonstrated that estimates of times to shared mtDNA haplotype ancestry were essentially a function of effective size of female populations, and that estimates of effective female population size ($N_{f(e)}$ value) for any sample or population could be derived from intrapopulational mtDNA diversities. The major assumptions were that mtDNA molecules are matriclinously inherited and that mtDNA variants are selectively neutral. Estimates of absolute $N_{f(e)}$ values require conversion to sidereal time using a presumed rate of mtDNA sequence evolution and an estimate of the generation time of a species (Avise 1992). Assuming that mtDNA sequence evolution and generation time are constant among geographic samples within species, the variation in intrapopulational mtDNA diversities among samples of red drum, black drum, and red snapper suggests differences in effective number of female parents or ancestors associated with individuals sampled among localities.

The differences in intrapopulational mtDNA diversities appear to vary geographically among the three species: in red and black drum, values are higher in the western Gulf and lower in the eastern Gulf, whereas the reverse was found in red snapper. These observed patterns may be important for two reasons. First, assuming spatial subdivision within the Gulf occurs in all three species, differences in intrapopulational mtDNA diversities may be useful in stock assessment. Secondly, the observation that differences in intrapopulational mtDNA diversities exist among samples within species, and that reverse geographic patterns occur among species, suggest that intrapopulational mtDNA diversities (and estimates of $N_{f(e)}$) may reflect contemporary as well as historical events.

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Population Structure of Eastern Oysters (*Crassostrea virginica*) Inhabiting the Laguna Madre, Texas, and Adjacent Bay Systems

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A survey of 16 enzyme systems and two structural proteins, among nine eastern oyster (*Crassostrea virginica*) populations in and adjacent to Laguna Madre, Texas, identified two genetically differentiated groups and the transition zone between them. The discontinuity in allele frequencies occurred between a reef in Corpus Christi Bay and reefs 26 km away in upper Laguna Madre. Although no fixed allelic differences were observed between populations from either side of the transition area, substantial frequency differences were observed at six gene loci, and both groups exhibited unique alleles. Spatial heterogeneity tests also suggested sizable allele frequency differences, as 8 of 15 polymorphic loci surveyed exhibited heterogeneity. Considerable genetic subdivision was observed, as 14 of 15 polymorphic loci surveyed exhibited significant fixation indices. The mean genetic similarity was 0.898 (range 0.813–0.977). Consequently, oysters inhabiting the Laguna Madre are genetically divergent from oysters inhabiting central and eastern Texas, the north-central and eastern Gulf of Mexico, and the western Atlantic coast. Thus, these groups should be considered as discrete management units, and the patterns of genetic exchange investigated. If eastern oysters inhabiting the Laguna Madre, Texas, are unique, they warrant protection.

Une étude de 16 systèmes enzymatiques et de deux protéines structurales dans neuf populations d'huîtres de l'Atlantique (*Crassostrea virginica*) dans la Laguna Madre, Texas, et dans la région avoisinante a permis d'identifier deux groupes génétiquement différents ainsi que la zone de transition entre les deux. Une discontinuité des fréquences alléliques est apparue entre un récif situé dans la baie de Corpus Christi et d'autres récifs situés à 26 kilomètres de distance dans la Laguna Madre supérieure. Bien qu'aucune différence allélique fixe n'ait été observée entre les populations des deux côtés de la zone de transition, des différences de fréquence substantielles ont été constatées dans le cas de six loci géniques et les deux groupes ont présenté des allèles uniques. Des tests d'hétérogénéité spatiale ont également indiqué des différences considérables des fréquences alléliques puisque 8 loci polymorphes sur les 15 étudiés ont présenté une hétérogénéité. Un fractionnement génétique considérable a été observé du fait que 14 loci polymorphes sur les 15 étudiés ont présenté des indices de fixation importants. La ressemblance génétique moyenne était de 0,898 (étendue de 0,813 à 0,977). Conséquemment, les huîtres vivant dans la Laguna Madre sont divergentes, génétiquement parlant, des huîtres vivant dans le centre et l'est du Texas, la partie centre-nord et l'est du golfe du Mexique et l'ouest de la côte Atlantique. Ainsi, ces groupes devraient être considérés comme des unités distinctes aux fins de la gestion et les modes d'échanges génétiques devraient être étudiés. Si les huîtres de l'Atlantique de la Laguna Madre, au Texas, sont uniques, leur protection serait justifiée.

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The eastern oyster, *Crassostrea virginica*, ranges from St. Lawrence Bay, Nova Scotia, through the Gulf of Mexico to the Yucatan Peninsula, Mexico, to the West Indies (Galstoff 1964), and may extend to Brazil (Gunter 1951). Throughout this vast range, genetic differentiation and nonheritable variation in morphology and physiology have been reported. Shell morphology has proven susceptible to environmental influences (Galtsoff 1964), but exhibits no predictive variation among geographic populations (Groue and Lester 1982). In contrast, differential spawning behavior in response to thermal stimuli (Stauber 1950) and variation in growth rates due to adaptation to hypersaline conditions (Breuer 1962) has resulted in identification of several physiological ecotypes. Population genetic surveys have indicated that eastern oysters comprise at least four geographic races (designated Canadian, U.S. Atlantic, U.S. Gulf coast, and

FIG. 1. General collecting localities for nine geographic populations of eastern oysters, *Crassostrea virginica*, from the central and southern Texas Gulf coast: (1) East Matagorda Bay (EMB), 2 km south of Boggy Bayou; (2) San Antonio Bay (SAB) at Welder Flats and the intracoastal waterway (ICWW); (3) Corpus Christi Bay (CCB) at Indian Point; (4) upper Laguna Madre 1 (ULM1) at Boat Hole near Demit Island; (5) upper Laguna Madre 2 (ULM2) approximately 2.5 km east of ICWW channel marker 27 near Dead Man's Hole; (6) lower Laguna Madre 1 (LLM1), 2 km west of ICWW channel marker 241 and approximately 20 km north of Port Mansfield, Texas; (7) lower Laguna Madre 2 (LLM2), approximately 5 km east of the Port Mansfield Harbor; (8) lower Laguna Madre 3 (LLM3), 2 km east of Medio Island and the ICWW, approximately 17 km north of Port Isabel, Texas; and (9) at the southwesternmost portion of South Bay (SB).

Bay of Campeche), that differ from the physiological ecotypes (Groue and Lester 1982; Buroker 1983; Hedgecock and Okazaki 1984; Karl and Avise 1992).

Within the northern Gulf of Mexico, oysters occupying lower Laguna Madre, Texas, have been reported to be physiologically (Breuer 1962) and genetically (Groue and Lester 1982; Buroker 1983) divergent from other populations. Oysters in the Laguna Madre are atypical in having adapted to hypersaline conditions (Breuer 1962). Mean annual rainfall in this semiarid estuary is approximately 64 cm; less than half the precipitation received along the upper Texas coast (Buroker 1983). The precipitation pattern, in association with a lack of major river inflow and increased evaporation corresponding to a decrease in latitude and an increase in insolation, results in a north–south salinity (and temperature) gradient along the Texas coast. Salinities in most Texas estuaries range from 5 to 30‰, while salinities in the Laguna Madre range from 35 to 60‰ (Diener 1975). The genetic differences distinguishing lower Laguna Madre oysters from other populations in the Gulf of Mexico may have evolved as a result of differential selection within this divergent habitat and/or through stochastic processes such as genetic drift of alleles (Groue and Lester 1982).

The eastern oyster population in South Bay is the only commercially exploited population in the Laguna Madre. South Bay experiences 35‰ salinity or higher for much of the year (Hook 1986), yet oysters spawn and thrive under these hypersaline conditions (Breuer 1962). The genetic relatedness of South Bay oysters to other established populations in lower Laguna Madre and the remainder of the Gulf of Mexico is unknown.

Oysters have not been harvested commercially from Corpus Christi Bay, Texas, since the decline of that fishery in the early 1960s (Martinez 1963; Gray et al. 1990). Reasons for the demise of this fishery are unknown, although habitat loss resulting from shell dredging and persistent high salinities from reduced freshwater inflow may be contributing factors. If high salinities are inhibiting Corpus Christi Bay oysters from attaining harvestable numbers, it may be possible to repopulate the bay by stocking animals adapted to hypersaline conditions similar to those now existing in Corpus Christi Bay. Research has been initiated to determine the feasiability of transplantation of South Bay oysters into Corpus Christi Bay (Gray et al. 1990).

Extensive transplantation of native oysters along major coasts has long been used to sustain fisheries without regard for adaptations of local races to new environments. However, if natural selection is the major mechanism maintaining the genetic differences between geographic populations, careful consideration should be given to any management plan incorporating transplantations. Information on population structure of oysters from South Bay and all of the Texas Gulf coast is crucial as agencies responsible for management of this resource have been charged with development of long-term management plans. An extensive allozyme survey could clearly delineate the population structure of eastern oysters in the Laguna Madre and adjacent bays and augment development of management plans.

The objective of this study was to expand on previous eastern oyster population surveys (Groue and Lester 1982; Buroker 1983) by describing the pattern of allelic variability among oyster populations inhabiting the Laguna Madre, Texas, and adjacent bay systems. Specifically, an extensive examination of allele frequencies among all known oyster populations in the Laguna Madre system and adjacent bays was performed to (1) identify the overall population structure,

TABLE 1. Enzyme and structural protein systems surveyed in Eastern oysters, *Crassostrea virginica*, from the central and southern Texas Gulf coast.

Enzyme or protein name (abbreviation)	Enzyme No.	No. of loci	Tissue[a] source	Buffer[b] system
Aspartate aminotransferase (*AAT*)	2.6.1.1	2	L	2
Acid phosphatase (*ACP*)	3.1.3.2	1	L	2
Aconitate hydratase (*ACO*)	4.2.1.3	1	L	1
Adenylate kinase (*AK*)	2.7.4.3	2	L	1
Alanine aminotransferase (*ALAT*)	2.6.1.2	1	M	2
Catalase (*CAT*)	1.11.1.6	1	M	2
Dihydrolipoamide dehydrogenase (*DLDH*)	1.8.1.4	1	L	2
Esterase[c] (*EST*)	-.-.-.-[d]	3	M, L	1
Fructose-biphosphate aldolase (*ALDO*)	4.1.2.13	1	L	2
Glyceraldehyde-3-phosphate dehydrogenase (*GAPD*)	1.2.1.12	1	M	2
Glucose-6-phosphate isomerase (*GPI*)	5.3.1.9	1	M	1
Isocitrate dehydrogenase (*IDHP*)	1.1.1.42	2	L	2
Malate dehydrogenase (*sMDH*)	1.1.1.37	2	L	2
Malic enzyme (*sMEP*)	1.1.1.40	1	L	2
Mannose-6-phosphate isomerase (*MPI*)	5.3.1.8	1	M	1
Cytosol aminopeptidase (*LAP*)	3.4.11.1	2	L	1
Peptidase-GL[e] (*PEP-GL*)	3.4.-.-	1	M	1
Phosphogluconate dehydrogenase (*PGDH*)	1.1.1.44	1	M	2
Phosphoglucomutase (*PGM*)	2.7.5.1	1	M	2
General protein (*PROT*)	-.-.-.-	2	M	2
Superoxide dismutase (*sSOD*)	1.15.1.1	1	L	2
Xanthine dehydrogenase (*XDH*)	1.1.1.204	1	L	1
Total		30		

[a]M, adductor muscle; L, liver.
[b]Gel buffer systems: 1, Tris borate EDTA, pH 8.0; 2, tris citrate, pH 7.0.
[c]*EST-1*, D, substrate was methylumbelliferyl acetate; *EST-2*, substrate was alpha-napthyl propionate.
[d]- represents nonspecific enzyme or protein.
[e]Substrate was glycyl-L-leucine.

(2) clearly delineate any areas of genetic discontinuity, and (3) determine the management implications of the observed population structure.

Materials and Methods

Eastern oysters were collected from January through July 1989 at nine localities (Fig. 1) along the central and southern Texas coast: (1) East Matagorda Bay (EMB), 2 km south of Boggy Bayou; (2) San Antonio Bay (SAB) at Welder Flats and the intracoastal waterway (ICWW); (3) Corpus Christi Bay (CCB) at Indian Point; (4) upper Laguna Madre 1 (ULM1) at Boat Hole near Demit Island; (5) upper Laguna Madre 2 (ULM2) approximately 2.5 km east of ICWW channel marker 27 near Dead Man's Hole; (6) lower Laguna Madre 1 (LLM1), 2 km west of ICWW channel marker 241 and approximately 20 km north of Port Mansfield, Texas; (7) lower Laguna Madre 2 (LLM2), approximately 5 km east of the Port Mansfield Harbor; (8) lower Laguna Madre 3 (LLM3), 2 km east of Medio Island and the ICWW, approximately 17 km north of Port Isabel, Texas; and (9) at the southwesternmost portion of South Bay (SB). Oysters were collected by dredge or hand. Live oysters were placed on ice and transported to the Perry R. Bass Marine Fisheries Research Station, Palacios, Texas, for processing. Total shell length (mm) was determined by measuring from the beak of the right valve to the furthest point on the shell margin. Only animals ≥60 mm were used due to evidence of size-specific variability (Koehn et al. 1973; Singh and Zouros 1978). Liver and muscle tissues were dissected, homogenized separately in an equivalent amount of buffer (0.01M Tris-HCl, 0.001M β-mercaptoethanol, 0.001M EDTA, pH 7.5; Selander et al. 1971), centrifuged for 15 min at 5000 rpm, and stored in liquid nitrogen or an ultracold freezer (−80°C) until electrophoretic examination.

Protein variation was assayed by vertical starch-gel electrophoresis (Morizot and Siciliano 1984). Gels were prepared as 12% suspensions of hydrolyzed starch (StarchArt Co., Smithville, Texas). Gel and electrode buffers utilized were Tris borate EDTA, pH 8.0, and Tris citrate, pH 7.0. Histochemical staining followed the methods of Selander et al. (1971), Ayala et al. (1974), Siciliano and Shaw (1976), and Harris and Hopkinson (1976). Electromorph nomenclature followed that of Shaklee et al. (1990), such that alleles were identified by sequential alphabetic codes.

BIOSYS-1 (Swofford and Selander 1981) was employed to ascertain allele frequencies, the proportion of loci heterozygous (*H*) in the average individual, the proportion of the loci polymorphic ($P_{0.95}$) for each population, deviations from expected Hardy–Weinberg genotypic proportions, and Rogers' (1972) genetic similarity (*S*) for all pairwise comparisons of populations. A dendogram produced from estimates of *S* using the unweighted pair-group method with arithmetic averaging (UPGMA) algorithm (Swofford and Selander 1981), provided a graphical representation of the relative similarity among populations. The effective number of alleles ($1/\Sigma$ (allele frequency)^2) was calculated for each locus in each population.

TABLE 2. Genic variation at six differentiated gene loci in eastern oysters, *Crassostrea virginica*, collected from nine populations along the Texas Gulf coast. A description of population acronyms is provided in the text. *H* is the proportion of individuals sampled that are actually heterozygous. *N* is the number of individuals sampled. Summary statistics (% polymorphic loci, effective alleles, and heterozygous loci/individual) are provided for all (15) polymorphic loci surveyed.

		Population								
Locus	Allele	EMB	SAB	CCB	ULM1	ULM2	LLM1	LLM2	LLM3	SB
EST-1	*a	0.808	0.870	0.797	0.233	0.061	0.052	0.012	0.067	0.041
	*b	0.192	0.130	0.203	0.663	0.841	0.813	0.869	0.758	0.862
	*c	0.000	0.000	0.000	0.105	0.098	0.127	0.113	0.158	0.097
	*d	0.000	0.000	0.000	0.000	0.000	0.007	0.006	0.017	0.000
	H	0.283	0.259	0.281	0.372	0.293	0.313	0.262	0.400	0.255
	N	60	54	96	43	82	67	84	60	98
EST-D	*a	0.592	0.713	0.672	0.128	0.043	0.052	0.030	0.083	0.020
	*b	0.408	0.278	0.313	0.872	0.957	0.925	0.940	0.908	0.969
	*c	0.000	0.009	0.016	0.000	0.000	0.022	0.018	0.000	0.010
	*d	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.008	0.000
	H	0.583	0.352	0.479	0.256	0.085	0.149	0.119	0.150	0.061
	N	60	54	96	43	82	67	84	60	98
GPI	*a	0.085	0.009	0.000	0.000	0.000	0.000	0.006	0.000	0.005
	*b	0.093	0.176	0.177	0.012	0.019	0.015	0.000	0.008	0.005
	*c	0.390	0.463	0.391	0.214	0.044	0.146	0.095	0.133	0.062
	*d	0.424	0.352	0.370	0.440	0.556	0.631	0.649	0.558	0.613
	*e	0.008	0.000	0.036	0.060	0.038	0.000	0.000	0.017	0.041
	*f	0.000	0.000	0.026	0.167	0.119	0.108	0.119	0.125	0.119
	*g	0.000	0.000	0.000	0.095	0.106	0.077	0.119	0.158	0.124
	*h	0.000	0.000	0.000	0.012	0.019	0.023	0.012	0.000	0.031
	H	0.695	0.537	0.625	0.667	0.563	0.569	0.548	0.583	0.629
	N	59	54	96	42	80	65	84	60	97
LAP-2	*a	0.000	0.000	0.000	0.100	0.018	0.038	0.036	0.052	0.051
	*b	0.000	0.000	0.000	0.244	0.177	0.114	0.143	0.217	0.117
	*c	0.000	0.000	0.036	0.344	0.451	0.500	0.488	0.433	0.454
	*d	0.050	0.028	0.094	0.178	0.110	0.189	0.131	0.167	0.235
	*e	0.283	0.231	0.365	0.111	0.171	0.091	0.125	0.083	0.107
	*f	0.408	0.454	0.359	0.022	0.073	0.068	0.077	0.058	0.036
	*g	0.208	0.204	0.135	0.000	0.000	0.000	0.000	0.000	0.000
	*h	0.050	0.083	0.010	0.000	0.000	0.000	0.000	0.000	0.000
	H	0.650	0.648	0.656	0.578	0.732	0.636	0.750	0.667	0.622
	N	60	54	96	45	82	66	84	60	98
sMDH-1	*a	0.992	1.000	0.917	0.384	0.185	0.149	0.107	0.133	0.122
	*b	0.008	0.000	0.083	0.616	0.815	0.851	0.893	0.867	0.878
	H	0.017	0.000	0.125	0.349	0.272	0.239	0.190	0.200	0.245
	N	60	54	96	43	81	67	84	60	98
PEP-GL	*a	0.008	0.009	0.000	0.012	0.000	0.000	0.000	0.000	0.005
	*b	0.100	0.056	0.021	0.012	0.068	0.000	0.006	0.017	0.026
	*c	0.142	0.222	0.189	0.291	0.216	0.054	0.066	0.067	0.072
	*d	0.117	0.120	0.211	0.477	0.574	0.800	0.765	0.683	0.753
	*e	0.442	0.454	0.458	0.140	0.117	0.077	0.090	0.133	0.077
	*f	0.183	0.139	0.084	0.035	0.025	0.046	0.054	0.075	0.041
	*g	0.008	0.000	0.037	0.035	0.000	0.023	0.018	0.025	0.021
	*h	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
	H	0.700	0.685	0.663	0.605	0.593	0.354	0.422	0.483	0.351
	N	60	54	95	43	81	65	83	60	97
% polymorphic loci ($P_{0.95}$)		0.500	0.458	0.500	0.500	0.417	0.542	0.458	0.458	0.458
Effective alleles		1.9	1.8	2.0	2.0	1.8	1.6	1.6	1.7	1.6
Heterozygous loci per individual (±SE)		0.226 (0.055)	0.200 (0.050)	0.224 (0.053)	0.215 (0.048)	0.195 (0.050)	0.168 (0.041)	0.173 (0.045)	0.182 (0.046)	0.170 (0.044)

Tests of allele frequency heterogeneity among all localities and between designated groupings were determined with log-likelihood ratios (*G*-tests) using BIOM-PC (Rohlf 1987). Genetic divergence among populations was further quantified with the *F*-statistics of Wright (1978); all *F*-statistics were generated with BIOSYS-1 (Swofford and Selander 1981).

TABLE 3. Log-likelihood G-values from contingency-table analysis of allele counts in eastern oysters, *Crassostrea virginica*, from nine localities along the Texas Gulf coast. G-values were compared with critical values of the χ^2 distribution with (populations -1) (alleles -1) degrees of freedom. Assigned groupings are abbreviated as follows: Gulf of Mexico (GOM; $N = 3$; EMB, SAB, CCB), Laguna Madre (LM; $N = 6$; ULM1-SB), upper Laguna Madre (ULM; $N = 2$; ULM1, ULM2), and lower Laguna Madre (LLM; $N = 4$; LLM1-3, SB). *Significant allele frequency heterogeneity ($P < 0.05$).

Locus	All samples	Within groups				Between groups			
		GOM	LM	ULM	LLM	GOM vs. LM	GOM vs. ULM	GOM vs. LLM	ULM vs. LLM
sAAT-1	14.33	2.21	2.80	0.00	2.25	2.15	2.55	2.15	0.18
mAAT-2	55.17*	8.75	27.10	8.33*	15.53	6.36	1.90	6.19	2.12
AK	49.93*	5.99	26.41	7.08	7.36	4.42	3.19	6.68	4.44
EST-1	498.02*	2.05	37.71*	12.58*	8.56	129.81*	106.28*	129.83*	6.52
EST-D	303.52*	5.95	19.90	5.30*	8.98	78.17*	65.59*	78.47*	3.08
GPI	345.78*	28.10*	47.12	14.60*	22.89	61.38*	82.24*	63.38*	7.00
mIDHP-1	19.97	8.64	7.57	0.26	5.51	1.01	1.30	1.01	0.30
IDHP-2	12.23	6.80	6.88	0.26	1.52	0.95	0.19	3.60	2.23
LAP-2	668.41*	22.82*	35.65	14.03*	12.32	146.12*	155.08*	152.86*	3.71
sMDH-1	633.49*	12.93*	30.68*	10.00*	0.77	150.35*	118.12*	169.74*	6.95*
sMDH-2	14.50	1.68	9.48	3.93	3.40	2.58	1.30	3.87	0.90
MPI-2	39.21	16.57*	20.53	3.22	16.75	1.64	1.77	2.61	0.58
PEP-GL	277.09*	20.81	62.54*	11.55	10.53	58.51*	45.64*	72.64*	17.33*
PGDH-A	32.96	6.91	26.24*	7.30	12.36	1.65	1.21	4.36	5.00
PGM-1	41.38	16.24	19.20	5.87	11.64	2.53	2.38	2.86	0.31

The fixation index (F_{ST}) was used to estimate the reduction in heterozygosity of a subpopulation due to population subdivision. A chi-square test was used to test the null hypothesis, $F_{ST} = 0$ (Workman and Niswander 1970). The inbreeding coefficient (F_{IS}), an indicator of nonrandom mating within subpopulations, was also determined. Random mating ($F_{IS} = 0$) was examined with a chi-square test as described by Li and Horvitz (1953).

Results

Genetic Variation

A survey of 16 enzyme systems and two structural proteins, in nine eastern oyster populations from the Texas Gulf coast, produced consistently scorable electromorphs for 24 putative gene loci (Table 1). Significant polymorphism ($P_{0.95}$) was detected in one or more populations at 15 loci; nine additional loci exhibited no discernable allelic variation. Detectable allozyme variation was observed in *ACP-2**, *ACO-1**, *ALAT-1**, *CAT-1**, *GAPD-1**, and *LAP-1**. However, these enzyme systems were not consistently resolvable in all samples and were eliminated from statistical analyses.

Preliminary examination of allelic frequencies revealed the presence of two genetically divergent groups of oyster populations with a major transition in genetic structure occurring between CCB and ULM1 (Table 2). Although no diagnostic alleles were observed between populations on either side of the transition area, substantial allele frequency differences were observed at six gene loci: *EST-1**, *EST-D**, *GPI**, *LAP-2**, *MDH-1**, and *PEP-GL**. In addition, unique alleles were observed at three loci. The *EST-1*c*, *GPI*f*, **g*, and **h*, and *LAP-2*a* and **b* alleles were unique to Laguna Madre populations; the *LAP-2*g* and **h* alleles were observed to be unique north of the transition zone. The CCB and ULM1 populations exhibited allele frequencies at some loci that appeared to be intermediate between the two groups.

Similar genetic variability estimates were observed within and between the two distinct groups of oyster populations. The proportion of polymorphic loci per population averaged 47.2% and ranged from 41.7% in oysters from ULM2 to 54.2% in specimens from LLM1 (Table 2). The average number of effective alleles per locus was slightly higher for populations north of the transition zone (1.91) than for the Laguna Madre populations (1.70). The average number of effective alleles ranged from 2.0 in CCB and ULM1 to 1.6 in LLM2; the average for all populations was 1.8 (Table 2). The percentage of heterozygous loci per individual (H) ranged from 16.8% in LLM1 oysters to 22.6% in oysters from EMB. Average H determined from all loci surveyed was 19.5%. A clinal pattern in H estimates among eastern oysters inhabiting the study area resulted in a statistically significant correlation between average individual H and degrees north latitude (i.e., H decreased with decreasing latitude; $r_S = 0.83$, $P < 0.005$). Reduction in single-locus heterozygosities for all Laguna Madre populations at *EST-D** and *PEP-GL** loci accounted for much of the observed decrease (Table 2). Genotypic frequencies deviated from Hardy–Weinberg equilibrium in only two instances: *LAP-2** in ULM1 ($\chi^2 = 105.23$, df = 15, $P < 0.0001$) and in SB ($\chi^2 = 28.87$, df = 15, $P < 0.02$).

Spatial heterogeneity test results corroborated the existence of substantial allele frequency differences among oyster populations, as eight of 15 polymorphic loci surveyed exhibited allele frequency heterogeneity (Table 3). The identification of a transition area provided a natural demarcation for establishing hierarchial geographic groupings to allow examination of within- and between-group allele frequency heterogeneity. The three populations north of the transition area (EMB, SAB, and CCB) were combined as the Gulf of Mexico (GOM) group, and the six remaining populations were merged as the Laguna Madre (LM) group. The LM group was further partitioned into upper Laguna Madre (ULM; comprising ULM1 and ULM2) and lower Laguna Madre (LLM; LLM1, LLM2, LLM3, and SB) subgroups.

Oysters comprising the GOM group exhibited significant allele frequency heterogeneity at *GPI**, *LAP-2**, *MDH-1**, and *MPI-2** loci (Table 3). The LM group exhibited

TABLE 4. *F*-statistics for eastern oysters, *Crassostrea virginica*, from nine localities along the Texas Gulf coast. *$P < 0.05$ for F_{IS} and $F_{ST} = 0$.

Locus	F_{IS}	χ^2 (df)	F_{ST}	χ^2 (df)
sAAT-1	−0.038	2.70 (6)	0.004	15.43 (24)
mAAT-2	0.080	12.33 (6)	0.040*	154.08 (24)
AK	0.121*	37.49 (10)	0.014*	71.90 (32)
EST-1	0.038	2.71 (6)	0.415*	1603.56 (24)
EST-D	−0.047	4.25 (6)	0.406*	1568.78 (24)
GPI	0.044	8.47 (28)	0.070*	624.26 (56)
mIDHP-1	−0.027	0.90 (3)	0.015*	38.58 (16)
IDHP-2	−0.038	1.80 (3)	0.023*	59.25 (16)
LAP-2	0.074	24.83 (28)	0.125*	1128.75 (56)
sMDH-1	0.108*	7.52 (1)	0.587*	754.88 (8)
sMDH-2	−0.031	1.93 (6)	0.010*	38.64 (24)
MPI-2	0.124*	49.51 (15)	0.013*	83.59 (40)
PEP-GL	0.044	8.49 (28)	0.161*	1438.05 (56)
PGDH-A	0.034	2.31 (6)	0.032*	123.46 (24)
PGM-1	−0.009	0.32 (15)	0.014*	89.74 (40)
Mean	0.061		0.164	

heterogeneous allele frequencies at the *EST-1**, *sMDH-1**, *PEP-GL**, and *PGDH-A** loci. Within the LM group, the ULM subgroup exhibited heterogeneity at the *mAAT-2**, *EST-1**, *EST-D**, *GPI**, *LAP-2**, and *sMDH-1** loci, while the LLM subgroup exhibited homogeneous allele frequencies at all loci. All between-group comparisons that bridged the transition area produced heterogeneous allele frequencies at the *EST-1**, *EST-D**, *GPI**, *LAP-2**, *MDH-1**, and *PEP-GL** loci. The comparison between ULM and LLM subgroups produced heterogeneous allele frequencies at *sMDH-1** and *PEP-GL** loci.

Random Mating

Inbreeding coefficients (F_{IS}) suggested modest deviation from panmixia among eastern oyster populations along the Texas Gulf coast (Table 4). The F_{IS} values ranged from −0.047 at *EST-D** to 0.124 at *MPI-2**. The mean F_{IS} value for all loci in all populations was 0.061. Statistically significant F_{IS} values were observed at *AK** (0.121), *sMDH-1** (0.108) and *MPI-2** (0.124) loci; all three values suggested an excess of homozygous genotypes. Homozygote excesses at ULM1 (0.230) and LLM1 (0.242) populations were contributors to the overall F_{IS} value at the *AK** locus. The overall F_{IS} value for *sMDH-1** was strongly influenced by homozygote excesses from CCB (0.182) and ULM1 (0.262) oysters, while ULM1 (0.222) and LLM1 (0.223) oysters contributed significantly to the overall F_{IS} value for *MPI-2**. Negative F_{IS} values observed for six loci suggested slight excesses of heterozygotes over those expected from a randomly mating population. The mean F_{IS} value for the GOM populations was 0.051, while mean F_{IS} for the LM populations was slightly higher at 0.066. After removal of the ULM populations, the level of inbreeding in LLM populations was identical to that observed in GOM populations (0.051).

Genetic Subdivision

Considerable genetic subdivision was observed among the eastern oyster populations inhabiting the entire study area. Fourteen of 15 polymorphic loci surveyed exhibited statistically significant F_{ST} values; only *sAAT-1** did not show significant population substructuring (Table 4). The estimated F_{ST} values ranged from 0.004 at *sAAT-1** to 0.587 at *sMDH-1**. The mean F_{ST} value for all loci among all populations was 0.164, a value consistent with high genetic differentiation of populations (Hartl 1981). The F_{ST} values for the six loci that exhibited major allele frequency differences between the two subpopulations contributed heavily. Considerable subdivision was indicated in comparisons between CCB and ULM1 populations (mean $F_{ST} = 0.095$) and among populations outside the transition area (EMB, SAB and LLM populations; mean $F_{ST} = 0.184$). However, only moderate population substructuring was indicated among the GOM populations (mean $F_{ST} = 0.010$) and among the LM populations (mean $F_{ST} = 0.022$). Little population substructuring was indicated among the four LLM populations (mean $F_{ST} = 0.007$).

Genetic Similarity

Estimates of Rogers' (1972) genetic similarity (S) among populations were generated for all surveyed loci. The overall S was 0.898, ranging from 0.813 between SAB and SB oysters to 0.977 between SB and LLM2 oysters. The mean S was 0.961 for EMB, SAB, and CCB oysters and 0.958 for the six Laguna Madre populations. The mean S for the four LLM populations was 0.973. The mean S between EMB, SAB, CCB, and the six Laguna Madre populations was 0.838.

A cluster analysis using the UPGMA algorithim applied to Rogers' S estimates, graphically illustrates the substantial genetic differentiation observed among the eastern oyster populations surveyed in this study (Fig. 2). Populations on either side of the transition area clustered together with the node separating the two distinct groups occurring at 0.837. The phenogram suggests that populations comprising the GOM group were genetically similar as were the populations comprising the LLM group. The phenogram also suggests that CCB and ULM1 (and perhaps ULM2) are transitional populations as they cluster at a lower similarity level than other populations in their respective groupings. The cophenetic correlation coefficient of the phenogram to the similarity matrix from which it was derived, was 0.980.

Discussion

An extensive allozyme survey of established eastern oyster populations (reefs) in and adjacent to the Laguna Madre, Texas, identified two genetically differentiated groups of populations with a transition zone between them. Discontinuity in allele frequencies occurred between a reef at Indian Point in northern Corpus Christi Bay and a reef in northernmost

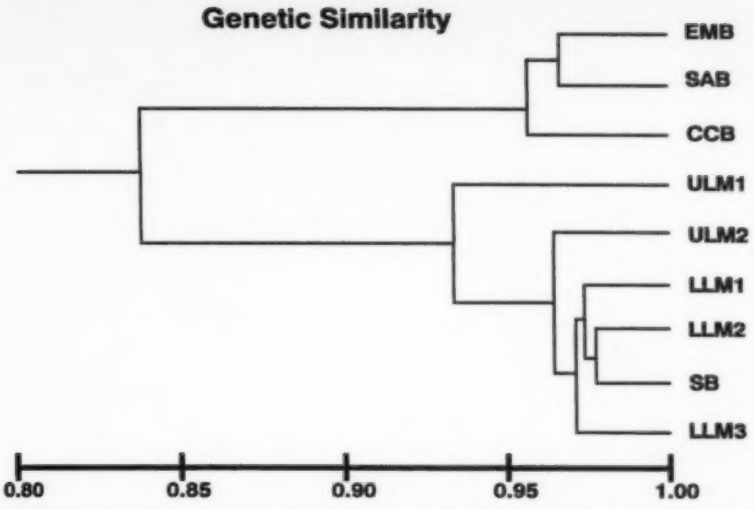

FIG. 2. Phenogram (UPGMA) depicting Roger's genetic similarity estimates (*S*) of allele frequencies in eastern oysters, *Crassostrea virginica*, from the Laguna Madre, Texas, and adjacent bay systems. The cophenetic correlation coefficient of the phenogram to the similarity matrix was 0.980.

upper Laguna Madre, at Boat Hole near Demit Island. This shift in genetic structure occurred between geographic populations separated by 26 km (nautical). Consequently, oysters inhabiting the Laguna Madre, Texas, are genetically divergent from oysters inhabiting central and eastern Texas, the north-central and eastern Gulf of Mexico, and the U.S. and Canadian Atlantic coasts. The upper Laguna Madre populations, while genetically distinct from eastern populations, were only slightly differentiated from the lower Laguna Madre populations (Fig. 2).

The conspicuous absence of some alleles suggested a limited amount of gene flow occurred between the two groups of populations. Numerous alleles of moderate frequency were observed to be unique to Laguna Madre oysters or were rare north of the transition zone. The *LAP-2*c* allele, which exhibited an average allele frequency of 45% in Laguna Madre populations, was found to be rare (4%, in CCB only) north of the transition zone (Table 2). The *EST-1*c*, *GPI*f*, **g*, and **h*, and *LAP-2*a* alleles exhibited a comparable pattern. Similarly, the *LAP-2*g* (18%) and **h* (5%) alleles were unique to populations north of the transition zone.

Prevailing currents are not conducive to providing an effective larval transport system in or out of the Laguna Madre. Thus, the potential for hydrographical barriers to gene flow does exist within this study area and it is probable this phenomenum has resulted in diminished gene exchange and contributed to the observed discontinuity in allele frequencies. However, given the eastern oyster's high individual fecundity and protracted planktonic dispersal stage, it seems plausible that some individuals would be carried the 26 km from northern Corpus Christi Bay to the upper Laguna Madre during the spawning season.

Regardless of the degree of isolation, effective gene flow between the two oyster subpopulations has been insufficient to overwhelm the effects on allelic divergence of genetic drift and/or natural selection. Differentiation among geographically proximal oyster populations could be attributed to a stochastic process that allowed random genetic drift to act upon the Laguna Madre populations. In natural populations, the capacity for genetic drift to impact allele frequencies is dependent on the effective population size (Hartl 1981). Owing to the finite size of eastern oyster reefs in the Laguna Madre and the limited gene exchange with eastern Gulf of Mexico oysters (assumed), allele frequencies at polymorphic loci would be expected to drift randomly. Conceivably, geographic isolation, drift, and a lack of sufficient gene flow could have resulted in divergent allele frequencies that have reached equilibrium (assumed). Alternatively, the allelic frequencies at any one or all of the six highly differentiated loci may have been maintained by natural selection in response to the large salinity and/or temperature differential between the Laguna Madre and estuaries of central and eastern Texas receiving more freshwater inflow. A large body of information implying an important role for natural selection in maintaining genetic differentiation between spatially proximal molluscan populations has been produced (Koehn 1991; Reeb and Avise 1990; Karl and Avise 1992).

Generally, the degree of genetic differentiation among aquatic organisms is associated with dispersal potential (Waples 1987). In the nearshore marine environment, where physical barriers are infrequent, dispersal potential should be great for a species with a planktonic life-history stage. Given the eastern oyster's high individual fecundity and protracted (2–3 wk) planktonic larval dispersal stage, the genetic differentiation observed in this study among spatially proximal populations was unexpected. Consequently, to provide the most efficacious management strategy for the oyster populations in Texas, the elements involved in maintaining the genetic differences should be ascertained. Determination of the mechanisms producing and/or maintaining the observed differentiation is beyond the scope of this manuscript. However, hydrographic isolation, genetic drift, and/or natural selection working independently or in synergy could account for these findings.

Management Implications

Variability in allele frequencies suggested two genetically distinct groups of eastern oyster populations inhabit the

Texas Gulf coast and allowed delineation of the transition zone between them. Information on population structure of eastern oysters along the Texas Gulf coast now exists to allow agencies responsible for management of this resource, to develop long-term management plans. Responsible management policy dictates that these groups be considered as discrete management units.

If natural selection is the major mechanism maintaining the genetic differences between these oyster subpopulations, careful consideration should be given to any management plan incorporating transplantations of oysters from one subpopulation to the other. By transplanting oysters across natural barriers (e.g., land barriers, ocean currents) man has provided many opportunities for parasites and diseases to find susceptible hosts. The Malpeque Bay disease in Prince Edward Island, Canada, the classical example of the consequences of mixing oyster races along a coast, followed transplantation of New England oysters (Andrews 1980). If the subpopulations identified in the present study have been genetically differentiated for centuries, careless transplantation of Laguna Madre oysters north of the transition zone (e.g., to Corpus Christi Bay) could jeopardize the oyster populations inhabiting the eastern Gulf of Mexico by introducing previously unknown diseases and/or pests and impacting the genetic integrity of the "race."

Texas Parks and Wildlife Department (TPWD) was granted a Coastal Preserve Lease for South Bay by the Texas General Land Office. The lease contains a requirement that TPWD develop a Comprehensive Management Plan describing proposed management goals and priorities for fish and wildlife species inhabiting the leased area. The allozyme survey reported here suggests that South Bay oysters are genetically similar to other oysters inhabiting the lower Laguna Madre. However, there is a distinct possibility that oyster populations of the Laguna Madre are a unique group of oysters. Further research should be conducted to determine the pattern of genetic exchange between Texas oysters and populations along the northern Mexico Gulf coast. If eastern oysters inhabiting the Laguna Madre, Texas, are unique, they should be protected.

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Gene Differentiation in Pacific Salmon (*Oncorhynchus* sp.): Facts and Models with Reference to Pink Salmon (*O. gorbuscha*)

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Analyzing population genetic data usually involves examining relationships among populations followed by analysis of the distribution of genetic variability. Genetic relationships are often depicted with multidimensional scaling or trees constructed from genetic distances; genetic variation within and among populations is partitioned using gene diversity measures such as F_{ST} or G_{ST}. Genetic distances or gene diversity are often used to estimate influences of gene drift, migration, and/or selection on observed gene differentiation. We used allozyme data for pink salmon populations to examine the theoretical models available for estimating magnitudes of these factors in Pacific salmon populations. The models included (1) mutation and gene drift; (2) mutation and migration; (3) migration and gene drift; and (4) gene drift, migration, and selection. These models suggest that gene drift and migration are probably important at the lowest levels of population hierarchy, but even very small forces such as weak heterogeneous selection and low migration levels may be important at higher levels. The accuracy of some estimates should be questioned because for many situations appropriate models are either not yet available or are not sufficiently refined. Also, the dynamic genetic structure of salmon populations makes it unlikely that the steady state assumed for many theoretical models has obtained.

En génétique des populations, l'analyse des données comprend habituellement une étude des relations entre les populations qui est suivie d'une analyse de la distribution de la variabilité génétique. Les relations génétiques sont souvent décrites au moyen d'analyses ou d'arbres multidimensionnels construits à partir des distances lociques; la variation génétique intra- et inter-population est cloisonnée par des mesures de diversité comme F_{ST} ou G_{ST}. Les distances lociques et la diversité génétique sont souvent employées pour estimer les effets de la dérive génique, de la migration ou de la sélection sur la différenciation génique observée. Nous avons utilisé des données sur les allozymes de populations de saumon rose pour examiner les modèles théoriques permettant d'estimer l'importance de ces facteurs chez les populations de saumon du Pacifique. Les modèles comprenaient : (1) la mutation et la dérive génique; (2) la mutation et la migration; (3) la migration et la dérive génique; et (4) la dérive génique, la migration et la sélection. Ces modèles laissent croire que la dérive génique et la migration jouent probablement un rôle important aux échelons inférieurs de la population, mais que même des forces très faibles comme une faible sélection hétérogène et de faibles niveaux de migration peuvent être importantes aux échelons supérieure. La justesse de certaines estimations pourrait être mise en doute parce que dans bon nombre de situations, on ne dispose par encore de modèles appropriès ou suffissamment perfectionnés. De plus, en raison de la structure génétique dynamique des populations de saumon, il est peu probable que l'état d'équilibre supposé dans bon nombre de modèles théoriques ait été atteint.

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The population genetics of Pacific salmon is in the phase of its development at which data on populations are being collected and the relationships among populations are being explored. In other words, the population genetics of salmon at this time deals primarily with identifying and ordering existing groupings in the multivariate space of gene or genotypic frequencies. The practical motivation for those studies has been to develop stock identification and separation methods that fishery managers can use to conserve, allocate, and manage salmon. Genetic studies have generally involved allozyme surveys to examine the potential for applications to stock identification or to develop baselines for use in addressing particular questions, although applications of DNA markers have begun.

Many genetic studies of salmonid populations have been conducted in the past two decades (e.g., pink salmon (*Oncorhynchus gorbuscha*): Aspinwall 1974; Johnson 1979; Gritsenko 1981; McGregor 1982; Altukhov et al. 1983, 1986; Beacham et al. 1985a, 1988b; Glubokovsky and Zhivotovsky 1986; Gagal'chy 1985; Gharrett et al. 1988; Zhivotovsky and Glubokovsky 1989; Glubokovsky et al. 1989; Zhivotovsky et al. 1989; Shaklee et al. 1991; Varnavskaya and Beacham 1992; Shaklee and Varnavskaya 1994; Noll et al. 1994; sockeye salmon (*O. nerka*): Altukhov et al. 1974a, 1974b; Grant et al. 1980; Utter et al. 1984; Wilmot and Burger 1985; Withler 1985; Kirpitchnikov 1979; Wood et al. 1994; Varnavsky and Everett 1994; Guthrie et al. 1994; chum salmon (*O. keta*); Kijima and Fujio 1979, 1982; Altukhov et al. 1980; Okazaki 1981, 1982; Beacham et al. 1985b, 1988a; Salmenkova et al. 1986; Phelps et al. 1994; Kondzela et al. 1994; Wilmot et al. 1994; chinook salmon (*O. tshawytscha*): Kristiansson and McIntyre 1976; Gharrett et al. 1987; Beacham et al. 1989; Utter et al. 1989; Bartley and Gall 1990; steelhead (*O. mykiss*): Allendorf 1975; Okazaki 1984; Reisenbichler and Phelps 1989; coho salmon (*O. kisutch*): Wehrhan and Powell 1987; masu salmon (*O. masou*): Okazaki 1986; multiple species: Utter et al. 1973, 1979; May et al. 1975; Reisenbichler and Phelps 1987). In many cases, the genetic marker surveys include native populations, and the data are amenable to population genetic analyses. The statistics ordinarily used to infer population structure from these data are genetic distances (Nei 1977) and gene diversities in terms of Wright's *F*-statistics (Chakraborty et al. 1982; Chakraborty and Leimar 1987; Weir 1990). Interpretations of such analyses are generally restricted to cluster analysis or classification of the collections analyzed. The explanation for the genetic differences observed among groups of collections is usually random genetic drift; that is, it is assumed that the alleles involved are neutral and that natural selection plays no role in the divergence observed. Other interpretations of the underlying causes of population structure are often ignored in these analyses.

Natural populations are not static. Many forces act on populations that can change genotypic frequencies (and potentially the baselines being developed for stock identification). It is important to be aware of the magnitude of changes in genetic structure that can result from gene flow among geographically separated regions or the directed selective pressures of fishery harvest methods, of enhancement practices, or environmental changes (natural or human). The purpose of this paper is to demonstrate that population-genetics data are often inadequate to distinguish among the causal factors of divergence. To estimate the magnitudes of factors that can generate divergence, different population genetic models that include different assumptions about the roles of the factors must be applied. In general, this approach must be taken in using either simple or complex models and in developing new models that are appropriate for interpreting data from real populations and population systems. Our approach deals with population genetics theory because we can see no other way to simultaneously consider the variety of models that reflect the different factors that can cause genetic divergence. We apply these models to a set of salmon data to demonstrate the kinds of conclusions that can be reached. In particular, we refer to pink salmon because this species has an interesting population structure and there are different opinions as to the causes of gene differentiation (Beacham et al. 1985a; Glubokovsky and Zhivotovsky 1986; Altukhov et al. 1983; Gharrett et al. 1988; Zhivotovsky and Glubokovsky 1989; Altukhov 1990; Varnavskaya and Beacham 1992). We use data available to us that include information on the same suite of allozyme loci (McGregor 1982; Gharrett et al. 1988; Lane et al. 1990; Noll et al. 1994).

Geographic and Temporal Structure of Pink Salmon

The pink salmon is the most numerous representative of Pacific salmon and accounts for about 60% (numerically) of all salmon caught commercially in Asia and North America (Heard 1991). Pink salmon inhabit the North Pacific Ocean and adjacent seas of the Arctic Ocean. On the Asian coast, natal streams range from the Korean Peninsula to the Chukotka Peninsula and occasionally the Lena River in the Arctic Ocean. On the North American coast, spawning pink salmon have been observed as far south as California and as far to the northeast as the Mackenzie River in Arctic Canada (McPhail and Lindsey 1970; Birman 1985). During their marine life, pink salmon are concentrated in the Pacific Ocean close to the South Kuril Islands, the Aleutian Islands, and the Sea of Japan (Takagi et al. 1981).

In their natural range, pink salmon have a rigid 2-yr anadromous life cycle; they spawn at 2 yr of age and die soon after spawning (Gilbert 1913; Davidson 1934; Rounsefell 1958). Because they are almost completely isolated reproductively, there are two genetically different lines of pink salmon, an odd-year line and even-year line that occupy the same general areas (Birman 1985; Heard 1991). Records of 1- or 3-yr-old pink salmon are rare (Anas 1959; Turner and Bilton 1968; Ivankov et al. 1975; Foster et al. 1980). There is some question about the accuracy of aging of some of the reported 3-yr-old fish (Bilton and Ricker 1965) and some of the 1-yr-old fish observed in streams near the nursery areas are immature. However, genetically similar even- and odd-year lines (Gharrett and Thomason 1987) resulted from a single transplant in the Great Lakes, which suggests that there are conditions under which 3-yr-old pink can occur naturally. The divergence of odd- and even-year lines is the most significant subdivision of pink salmon, that is, the highest level in the pink salmon population hierarchical structure.

Within each of the odd- and even-year lines exist geographically based groupings of pink salmon from which other hierarchical levels in population structure can be identified. For example, populations may be classified by continent of origin, geographical region within a continent,

drainage within a region, and so on down to (in theory) an individual redd. In addition to geographical hierarchy, there may also be temporal hierarchy in population structure (early and late races) as observed in the even- and odd-year runs in a stream (Taylor 1980; Ivankov 1986; Smoker et al. 1995). The hierarchy of pink salmon population structure has been examined previously for Asian (Zhivotovsky et al. 1989), Canadian (Beacham et al. 1985a, 1988b), and western Alaskan (Gharrett et al. 1988) populations.

The levels of hierarchy we will examine here are (1) the within-stream temporal structure observed for Auke Creek, near Juneau, Alaska, for 1979 and 1980 brood years (McGregor 1982); (2) among even-year populations in Southeast Alaska (McGregor 1982) and among even-year northwestern Alaskan populations (Gharrett et al. 1988); (3) among these regions and Hokkaido Island, Japan (Noll et al. 1994); (4) between even-year North American and Asian collections; and (5) between the aggregate of even-year collections and the single odd-year collection.

The data used in the analyses are for the presumed loci: *AAT-3**, *ADA-2**, *mAH-3**, *mAH-4**, *CK-A*1*, *CK-A2**, *G3PDH-1**, *GPI-B1,2**, *GPI-A**, *LDH-A1**, *LDH-A2**, *LDH-B2**, *LDH-C**, *sMDH-A1,2**, *sMDH-B1,2**, *mMEP-1**, *MPI**, *PEPD-1**, *PEPD-2**, and *sSOD-1**. Data were available for all of these loci in all collections.

The purpose of this analysis is not to analyze thoroughly pink salmon population structure. The data are incomplete both with respect to the number of collections included and the number of loci used. Rather, the purpose is to examine the models available for analyzing population structure and to obtain estimates of the magnitudes of the factors required if they are to play a significant role in determining population structure. From this, it will be possible to identify the kinds of experiments or data that will be required to understand the genetic structure of salmon populations.

Models

In the analysis of population genetics of salmonids, we are most concerned with the dynamic aspects of population genetic structure. The main factors that can cause or eliminate the genetic differentiation among populations are mutation, random gene drift, gene migration, and selection. The general dynamic theory of gene frequencies under these forces is quite advanced. The mathematical procedures available include dynamic models for estimating the direction and magnitude of the forces, although special models for particular population structures need to be developed in detail and there are problems with interpretation of data (Nei 1975; Manly 1985; Gillespie 1991; Zhivotovsky 1991; Nagylaki 1992). In this section, we consider some models that might be useful for the analysis of the dynamics of salmon population genetics.

Mutation Models

Substitution of one or more DNA base pairs, deletions or insertions of DNA sequences, transposition or reciprocal or nonreciprocal recombinations, and so forth may introduce new allelic variation into a population. The influences of these changes on the genetic differentiation among populations may be considered in terms of mutation models. (We are not considering horizontal transmission.) The theory of genetic distances developed by Nei (1972, 1975) considers the differences between populations that result from unique neutral substitutions of genes. The parameters that can be estimated depend upon whether or not migration is incorporated into the model.

Mutation – gene drift

The theory of genetic distances developed by Nei (1972) and Nei and Roychoudhry (1974), allows estimation of the time of divergence of two isolated populations, t (in years), under gene drift and neutral mutation using a rate of 10^{-7} suggested for electrophoretically distinguishable alleles (Nei 1987):

$$(1) \qquad t \approx 5 \times 10^6 D$$

where $D = -\ln I$ is Nei's standard genetic distance and $I = J_{XY}/(J_X J_Y)^{0.5}$ is the normalized gene identity estimated from genetic data. For electrophoretic data, this formula holds if the genetic identity, I, exceeds 0.82 (Nei 1987).

This model requires some assumptions; in particular, the populations must be at equilibrium with constant gene identities, J_X and J_Y, and no migration between them (Nei 1987). It is not possible to check the first assumption directly. However, if the populations were very small some time ago, t may be considered as an overestimate of the divergence time. As for the second assumption, even low levels of migration can dramatically reduce differences between populations caused by new neutral mutations. Because of that, the value of t will be underestimated. Hence, the estimating procedure for the divergence time depends on and is very sensitive to the population numbers and gene migration rates. Also, Equation (1) assumes that the average rate of mutations changing the electrophoretic mobility of the protein product is 10^{-7}. If the average substitution rate, α, is different for salmonids, equation (1) should be modified by $t = D/2\alpha$ (Nei 1987). Moreover, Equation (1) is valid only at the beginning of the divergence (when $t = 0$), when the two populations are genetically identical (Nei 1987). Of course, it is not possible to estimate or assume anything about the original state.

Estimates of divergence times between early- and late-run Auke Creek fish (Table 1) range between 1000 and 4000 generations and are comparable to the within-regional divergence times. These estimates hold only if there is neither gene flow between these runs nor among populations within the regions. Also it unlikely that these point estimates have much meaning because the genetic distances to which they correspond are quite small and, therefore, sensitive to sampling error. The estimated divergence times at higher levels of the hierarchy are about 12 000 years between North American regions, about 18 000 between Asian and North American fish, and about 18 000 years between even- and odd-year broods. The Asian pink salmon included were from a stream draining to the Okhotsk Sea. These estimates suggest that the divergence between Alaskan regions and between even- and odd-years date back to the late Pleistocene. The low water levels of the Wisconsin cold period may have separated the Okhotsk Sea from the Bering refuge and the Bering refuge from the southern refuge. It is likely, however, that estimates of divergence time at higher levels of hierarchy are very low because of gene flow.

Mutation–migration

The previous model holds only if migration rates are negligible relative to the mutation rate. For instance, Equation (1)

TABLE 1. Parameters for genetic comparisons at different levels of hierarchical structure of pink salmon populations. Estimates for characteristics at each level of hierarchy are: the effective population size (N_e); Nei's standard genetic distance (D); divergence time (t) in years, which results from accumulation of neutral mutations and gene drift; the migration rate at equilibrium if only mutation and migration act on the population (m_μ); the gene diversity relative to the total variability (G_{ST}); the average number of migrants per generation between subdivisions ($N_e m$); the average immigration rate for subdivisions (m); and the half-time to equilibrium (t_{eq}) in generations for the gene drift – migration process. N_e for each hierarchical level was determined from available catch data (Rigby et al. 1991) or escapement estimates (Eggers et al. 1993) for the weakest returns between 1960 and 1980. This time span includes many of the lowest returns in recent times. N_e based on catches assumed an exploitation rate of 60%, which is generally realized only for large returns; escapement indices tend to be underestimates (R. Marshall, Alaska Department of Fish. and Game, Juneau, AK 99824, personal communication; O. Mathisen, University of Alaska, Fairbanks, Juneau, AK 99801, personal communication).

Hierarchical level	Description	log(N_e)	D	Divergence time (t)	m_μ ($\times 10^5$)	G_{ST}	$N_e m$	m	t_{eq}
1st	Between runs within Auke creek (odd)	2–3	0.0008	4 000	45	0.005	50	0.5–5	1–10
	Between runs within Auke creek (even)	2–3	0.0002	1 000	12	0.0015	165	0.2–1	0.3–3
2nd	Among SE Alaska populations (even)	2–4	0.0007	3 500	17	0.006	41	$0.4–41\times10^{-2}$	1.2–120
	Among E. Bering Sea populations (even)	2–4	0.0009	4 500	11	0.008	31	$0.3–31\times10^{-2}$	1.6–160
3rd	Between SE Alaska and E. Bering Sea (even)	6–7	0.0024	12 000	4.6	0.018	3	$3.4–34\times10^{-7}$	$1.4–14\times10^{5}$
4th	Between Asia and N. America (even)	7–8	0.0035	17 700	3.7	0.023	3	$2.7–27\times10^{-8}$	$1.7–17\times10^{6}$
5th	Even-year and odd-year broods	7–8	0.0036	17 800	3.4	0.027	2	$2.2–22\times10^{-8}$	$2.0–20\times10^{6}$

is based on mutation rates of the order 10^{-7} per locus, so this model requires migration rates much less than 10^{-7} to be valid. If the magnitude of the migration rate, m_μ, is small, but much larger than the mutation rate, α, then the genetic identity eventually approaches an equilibrium value

$$(2) \qquad I = \frac{m_\mu}{m_\mu + \alpha}$$

(Nei and Feldman 1972; Nei 1987). Therefore, given the mutation rate and the estimated genetic identity, migration rates can be estimated as

$$(3) \qquad m_\mu = \frac{\alpha I}{1 - I}$$

and substituting a mutation rate of 10^{-7} for electrophoretic data (Nei 1987):

$$(4) \qquad m_\mu = 10^{-7}\frac{I}{1 - I}$$

The rates of migration estimated for this model are of the order of 10^{-4}–10^{-5}, which are far too small to be realistic for local populations and indicate that the populations are not in equilibrium with respect to mutation and migration, or that natural selection is involved. It should be emphasized that Equation (2) is the island model, without spatial or temporal structure, and therefore the parameter m is only a preliminary or conditional estimate of actual migration rates between subpopulations.

Migration – Gene Drift Model

It is unlikely that salmon populations are completely isolated; some genetic exchange or immigration occurs in most anadromous populations. The populations form a system (of subpopulations) related by gene flow. One measure of gene differentiation among the populations is the well known Wright's F-statistic and its generalizations (Wright 1969; Nei 1977; Weir 1990). We will refer to G_{ST}-statistics (Chakraborty et al. 1982) that are used in population studies, although these estimates are biased and depend both on the number of subpopulations and their sizes (Cockerham and Weir 1993). An unbiased estimate of G_{ST}, β, is given by Cockerham and Weir (1993), but it was developed only for a one-way classification and not generalized for hierarchically structured populations.

Of the dynamic models developed to apply G_{ST} when populations are related by migration, the best known is Wright's (1943) island model in which gene differentiation among populations results from random gene drift and homogenization results from immigrants from a large external population. Recently, this model has been generalized to include hierarchically structured populations with different migration rates at different levels of the hierarchy (Chakraborty and Leimar 1987; Slatkin and Voelm 1991). It was shown for a two-level hierarchical model consisting of g major groups within each of which there are k subpopulations that at equilibrium, gene differentiation (G_{ST}) at the lowest level of the hierarchy (among the subpopulations within the major groups) estimated by the Nei–Chakraborty measure eventually approaches the well known value

$$(5) \qquad G_{ST} \approx \frac{1}{1 + 4N_e m}$$

where N_e is the effective size of the subpopulations, m is the fraction of migrants coming into each of the subpopulations every generation. We can use Equation (2) to estimate the movement of migrants among subpopulations:

$$(6) \qquad N_e m \approx \frac{1 - G_{ST}}{4G_{ST}}$$

To be exact, gene differentiation among a finite number of subpopulations is

$$(7) \quad G_{ST} \approx \frac{1}{1+4N_e m[(n/n-1)]^2}$$

where n is the number of subpopulations within major groups (Takahata and Nei 1984; Slatkin and Voelm 1991). Hence

$$(8) \quad N_e m \approx \frac{1-G_{ST}}{4G_{ST}[n/(n-1)]^2}$$

Usually n is large enough at the lowest levels of hierarchy that Equation (3) holds.

At the same time, gene differentiation among the major groups (G_{GT}) estimated by the same measure (G_{ST}) eventually approaches

$$(9) \quad G_{GT} \approx \frac{1}{1+4N_{G_e} m_G (g/g-1)^2}$$

where m_G is the proportion of immigrants into each of the major groups each generation, and

$$(10) \quad N_{G_e} \approx \frac{kN_e}{1-G_{ST}}$$

is the effective size of the major groups and g is the number of major groups and k is the number of subpopulations forming each major group. If G_{ST} is small enough, as for pink salmon, the total size of all the subpopulations forming a major group can be estimated as kN_e. Thus, the gene differentiation at higher levels of the population hierarchy is determined by population sizes much greater than those at lower levels. G_{GT} also depends on the number of major groups. In particular, when there are two major groups

$$(11) \quad G_{GT} \approx \frac{1}{1+16N_{G_e} m_G}$$

If the number of major groups is large:

$$(12) \quad G_{GT} \approx \frac{1}{1+4N_{G_e} m_G}$$

as for G_{ST}. The above equilibrium values are nearly independent of mutation rate (Chakraborty and Leimar 1987). Although the result is obtained for a two-level subdivided population, it appears to be applicable to multiple-level hierarchies.

Thus, if one estimates gene differentiation among populations and does not neglect gene flow, it is more appropriate to use the above equations for estimating the number of migrants among groupings at the level of the hierarchy:

$$(13) \quad N_{G_e} m_G \approx \left(\frac{g-1}{g}\right)^2 \left(\frac{1-G_{GT}}{4G_{GT}}\right)$$

In particular, if there are only two major groups:

$$(14) \quad N_{G_e} m_G \approx \frac{1-G_{GT}}{16G_{GT}}$$

and if the number of major groups is large:

$$(15) \quad N_{G_e} m_G \approx \frac{1-G_{GT}}{4G_{GT}}$$

If we estimate $N_e m$ from the above equations and are able to estimate effective numbers from independent data, we can estimate m. Estimates of the ratio of effective number to census number range from 0.1 to 0.6; however, the average effective number over time is approximately the harmonic mean and because the dynamics of pink salmon populations are fluctuating dramatically, the numbers in Table 1 probably over estimate the average effective population size over recent millennia.

From the data that we are considering, gene flow estimates at the local and regional level are large both for the number of immigrants, $N_e m$, and the rate of migration, m. At the higher levels of hierarchy, however, estimates of the average number of migrants is about two or three, but estimates of migration rates are quite small, on the order of 10^{-6} to 10^{-8}; these would be larger if N_e were smaller.

It must be emphasized that these formulae hold only if the population is at equilibrium between gene drift as a diversifying factor and migration as an homogenizing factor. Without knowledge of the initial state of the system, whether it was homogeneous or heterogeneous, it is not possible to know if these expressions overestimate or underestimate gene flow. For one-level structured populations, approximate estimates of the half-time, t_{eq} (in generations), needed to approach equilibrium (Chakraborty and Leimar 1987) can be obtained from

$$(16) \quad \frac{1}{t_{eq}} \ll \frac{1}{2N_e} + 2m$$

Such estimates can also be applied to hierarchically structured populations where N_e and m are the effective size and migration rate among major groups at a given level. The estimates of half-time to equilibrium are quite low within regions but much larger between regions, 1.4 to 14×10^5 generations, and very large between continents and between brood years. In fact the time to equilibrium at the top levels of hierarchy suggests that equilibrium can not have been obtained. It is important to note that neither the migration – gene drift models nor the mutation–migration models are applicable if selection, either direct or indirect, acts on the loci.

Gene Drift – Migration – Selection Models

Most of the population genetic data available come from allozyme surveys. Nevertheless, there have been few breeding studies to confirm Mendelian inheritance, and those experiments ordinarily examine relatively few offspring, or pool families for incubation and subsequent analysis. Small numbers of progeny limit the statistical power, and pooled families may mask errors in parent typing and obscure individual family effects. As a result, very few data are available for examining genotypic ratios that may reflect selection. A compilation of the available data for individual families of pink salmon (Table 2) indicates that if directional selection toward the common homozygous type exists, it is weak because segregation does not differ significantly from a ratio of 1:1.

However, even very weak selection can cause substantial changes in populations over many generations. The segregation data estimate selection at an early life stage (among fry) and do not exclude the possibility of selection during early marine life when mortality is very high. Selection can change direction and magnitude during the life cycle, especially for a species that encounters varying environmental conditions. Unfortunately, there are no studies of life-history

TABLE 2. Summary of test crosses for segregation of allozymic alleles in individual families of pink salmon. Only crosses in which the homozygous parent carries the most common allele at the locus are included. Data are from Johnson (1979), McGregor (1982), and unpublished data (C.D. Russell, S.M. Kelley, B.L. Carney, A.N. Smoker, and A.J. Gharrett).

Locus	No. of crosses	Homozygote excess	Heterozygote excess	Total offspring	Goodness-of-fit test χ^2 (1 df)	Homo./het. ratio	Heterogeneity among crosses		
							G	df	*P*
*G3PDH-1**	13[a]	7	5	864	0.91	1.07	17.64	12	0.13
*mMEP-1**	7	6	1	587	2.86	1.15	3.32	6	0.76
*PGM-2**	3	0	3	180	6.42[b]	1.46	0.48	2	0.79
*sMDH-B1,2**	5	2	3	587	0.21	0.96	9.06	4	0.06
*sAAT-3**	10	4	4	591	0.29	1.04	6.62	9	0.68
*GPI-B1,2**	4	2	1	838	0.17	1.16	2.18	3	0.53
*PEPLT-1**	4	1	3	285	2.10	0.90	2.10	3	0.55
*PGDH**	7	1	4	435	0.39	0.94	4.07	6	0.67
*mAH-4**	2	1	1	173	1.67	1.21	0.99	1	0.32
*ADA-2**	5	4	0	279	3.44	1.25	0.76	4	0.94
*PEPD-2**	8	1	6	463	0.95	0.91	2.84	7	0.90
Total	68	29	31	5282	2.55	1.04	65.67	67	0.52

[a]One significant test ($P < 0.05$).
[b]$P = 0.011$.

related changes in selection at a locus, and such studies would be difficult to execute.

Homogeneous selection

Wright (1969) introduced a general model of gene differentiation under genetic drift, migration, and homogeneous selection, that is, selection whose strength and direction are the same for all subpopulations of the population system. He demonstrated that when heterozygotes had an advantage, at equilibrium the value of F_{ST} for a one-level structured population depends on both migration rate and intensity of selection:

$$(17) \quad F_{ST} = \frac{V_p}{\bar{p}(1-\bar{p})}$$

Equation (17) is a measure of the differentiation based on frequencies of a single allele, *A*, where *V* is the variance of the frequencies among subpopulations and $\bar{p}$ is the average of the allele frequencies. For any $N_e m \gg 1$, weak selection, and low migration, a relatively simple approximation of Wright's formula for F_{ST} in Equation 17 is

$$(18) \quad F_{ST} = \frac{1}{4N_e(m + (s+t)\bar{p}(1-\bar{p}))}$$

where *s* and *t* are selection coefficients against *AA* homozygotes and genotypes not possessing the *A* allele (Zhivotovsky and Feldman 1993). The Nei–Chakraborty measure, G_{ST}, coincides with F_{ST} when it operates with frequencies of a single allele.

Equation (18) shows that homogeneous selection acts like migration in the sense that it tends to make subpopulations become similar in allele frequencies. By introducing a generalized parameter

$$(19) \quad M_e = N_e\,(m + (s+t)\,\bar{p}(1-\bar{p}))$$

as a measure of the total action of homogenizing forces, we see that Equation (5) estimates M_e rather than $N_e m$ if homogeneous selection acts in the populations. Of course, Equations (7) and (8) also estimate this generalized parameter, not $N_e m$, for the higher levels of population hierarchy. Clearly even low levels of selection (of the same order as *m*) can be important in structuring the higher levels of hierarchy.

Heterogeneous selection

It is more likely that, if selection acts in a population, it will vary with ecological conditions. Indeed, because mortality in the redds and during early ocean life seem to be crucial in the overall survival of pink salmon, the local conditions result in selective differences among populations or regions. If such is the case, a model that incorporates such heterogeneous selection among subdivisions is more appropriate for explaining population structure than one that considers only gene flow and gene drift. Moreover, because heterogeneous selection can play a role as a diversifying factor (unlike homogeneous selection), the interaction between gene drift and heterogeneous selection and between gene flow and heterogeneous selection will eventually determine G_{ST} or F_{ST}.

At this time, deterministic models for the dynamics of F_{ST} have been developed for only some special cases (Zhivotovsky and Feldman 1993). Under both weak selection and low migration, the eventual gene differentiation depends on so-called migratory liabilities:

$$(20) \quad \theta_i = \frac{m_i}{m_i + (s_i + t_i)p_i(1-p_i)}$$

where *s*, *t*, and *p* are the same as defined above and the subscript *i* refers to the *i*th subpopulation. Here m_i is the immigration rate into the *i*th subpopulation. Because Equation (11) can be written

$$(21) \quad \theta_i = \frac{1}{1 + ((s_i + t_i)/m_i)p_i(1-p_i)}$$

it can be seen that the ratio of the selection coefficients to the immigration rate for each subpopulation, rather than their magnitudes is the primary dynamic parameter for this model.

The model was developed for arbitrary migration rates, m_{ij}, from the *j*th into the *i*th subpopulation. Although the expressions involving allele frequencies are too cumbersome for interpreting gene differentiation among subpopulations,

a relatively simple result is possible for a one-level subdivided population. At equilibrium between heterogeneous selection and migration:

$$(22) \quad F_{ST} \approx (1 - \bar{\theta})^2 F_{ecol}$$

where $\bar{\theta}$ is an average of θs and F_{ecol} is the value of F_{ST} that would result eventually in the population if there were no gene flow and gene differentiation resulted from the heterogeneous selection due to local environmental differences.

Although F_{ecol} is an abstract parameter, the notion can be useful. The ratio of F_{ST}/F_{ecol} is a measure of the relative influences of migration and heterogeneous selection on gene differentiation and can be measured, in principle, by the ratio of the strength of selection and migration:

$$(23) \quad \frac{F_{ST}}{F_{ecol}} \approx (1 - \bar{\theta})^2$$

It should be mentioned that Equation (22) holds for positive values of s and t. For arbitrary selection coefficients, the corresponding expression is too cumbersome, although the main conclusions are valid: the ratios of selection coefficients and migration rates determine the eventual gene differentiation among related populations (Zhivotovsky and Feldman 1993). Moreover, even if selection eliminates an allele in one population, gene flow will restore it from the rest of the population system.

It follows from Equation (13) that the observed range of differentiation in a structured population may be large if ecological differences, which lead to differences in selection coefficients among the subpopulations, are great enough to produce large F_{ecol} values and the ratio of selection and migration rates (s/m) is large. Conversely, little differentiation may be observed if the (s/m) ratio is small and ecological conditions not too diverse (F_{ecol} is small). From this perspective the difference between North American and Asian pink salmon, as well as regional differences, may be determined by selection even if it is very weak. The differences between the even- and odd-year lines can be explained by the slightly different selection regimes to which these lines have been exposed (V.S. Kirpitchnikov (deceased); Institute of Cytology, Russian Academy of Sciences, St. Petersburg, personal communication) or by differential contributions into migrant pools from the different regional assemblages (Zhivotovsky and Glubokovsky 1989; Zhivotovsky and Feldman 1993). The low heterogeneity within regions and between early and late runs, may also be influenced by low ecological diversity, larger migration rates, or both.

We are not aware of any model that simultaneously considers the effects of gene drift, gene flow, and heterogeneous selection on gene differentiation.

Discussion

We have presented several models for interpreting data acquired in population genetics surveys of anadromous Pacific salmon. The underlying question is: To what extent do gene drift, gene flow, and selection determine population structure? The general conclusion is that, from available data and population genetics models, nothing definite can be concluded about the relative contribution of these processes. Two important factors that affect our ability to reach conclusions are the insufficiency of data for both North American and Asian populations, and the very complex population dynamics of pink salmon. Work is presently under way to extend the genetic data base for pink salmon populations, but the theory of population and genetic dynamics of pink salmon is not sufficiently advanced to permit interpretation of population genetics data. The purpose of this paper, therefore, is to point out some of the misapplications of population genetics theory, and to identify some general questions in pink salmon biology that require answers before population genetic data can be correctly interpreted.

All of the available population genetics models include simplifying assumptions that restrict their scope of application. As a result, no data set should be interpreted using only a single model. For example, the same degree of genetic differentiation can result from genetic drift or weak heterogeneous (local) selection. Another example is that statistically significant divergence of neutral alleles can occur in the presence of gene flow although such differences are sometimes interpreted as the absence of gene flow (e.g., Varnavskaya and Beacham 1992).

Conclusion 1

In pink salmon, the presence of genetic differences between collections or groups of collections is not, by itself, evidence of the absence of genetically important gene flow among these groups. Such information must be considered together with independent complementary data, for example, from tagging or from ecological comparisons of the stream and marine conditions. The combination of these data may allow such conclusions. We should emphasize that genetic tagging is essential for accurate estimates of gene flow, because physically tagged fish may not (for behavioral, ecological, or genetic reasons) contribute to the gene pool to the extent that their physical numbers indicate (Gharrett and Seeb 1990). Physical tags document the movement of fish, but do not measure the extent of genetic exchange that results. Weirs and coastal traps exacerbate the problem because salmon, particularly pink salmon, may range over a broad area probing several streams before they move to a stream to spawn (Hoffman et al. 1984). In general, straying among nearby streams as measured by tagging is less than 10% (Heard 1991), but in some locations or instances may be much higher (D. Sharp, Alaska Department of Fish and Game, Cordova, AK 99574, personal communication). Because the data from physical tagging has relatively low precision, gene flow can not be precluded from tagging data. Also, occasional rare but large migrations may occur between populations under unusual environmental conditions (oil pollution, blocked streams, cold water barriers in open seas, etc.). Such movements tend to homogenize the populations. The persistence of the effect in the populations depends on the strength of the diversifying factors influencing the population structure. The tagging data available indicate that populations are not completely isolated, and are connected by at least low levels of gene flow either directly or through a chain of intermediate populations. It is also possible that there may be a low level of gene flow between brood years if marginal environmental conditions at the extremes of the range result in some 3-yr-old fish. If gene flow is important, it is natural to assume that it is not spatially or temporally constant and may fluctuate so that the stocks may be isolated from each other for several generations and the direction of flow may vary. This idea was used to formulate a concept of "fluctuating stocks" of pink salmon

(Glubokovsky and Zhivotovsky 1986). It is probable that even rare, but substantial, gene migrations can influence gene differentiation dramatically.

Conclusion 2

The mathematical theory of fluctuating gene flow must be further developed in order to understand the process and obtain estimates. It is important to obtain solutions to dynamic equations because it is unlikely that any of the differentiating process are in equilibrium for Pacific salmon.

In salmon populations, especially at the higher levels of pink salmon population hierarchy, gene drift is very slow, although small local populations and populations that experience environmental extremes may experience faster gene drift. In general, if selection coefficients and migration rates are much bigger than $1/N_e$, heterogeneous selection and gene flow should play a leading role in gene differentiation at these levels. For pink salmon whose regional stocks can reach 10^5 to 10^7, it means that selection and migration, even with magnitudes of the order of 10^{-5} to 10^{-3}, rather than gene drift, are the main factors.

The analysis of segregation of alleles (Table 2) indicates that if selection acts on those families, it is weak. However, the level of resolution is quite low. To detect statistically significant estimates of selection coefficients even as large a 0.01 requires that tens of thousands of progeny be examined for each pair of segregating alleles. It is also likely that selection acts not just at the earliest stage of development, but at later life stages. To estimate selection, it is necessary to analyze many large families and to compare carefully the distributions of genotypes at several life-history stages. Such data must be carefully interpreted because genotypic fitness may vary temporally and spatially, and selection is most likely to occur during stress (Hoffman and Parsons 1991; Hartl et al. 1985). Such sporadic selection may be important even if it does not occur every generation. Models need to be developed that consider fluctuating selection.

Geneticists used to consider that weak forces had little influence on the genetic structure of populations. However, biological systems may be sensitive to them. For example, Ricker's equation (e.g., Ricker 1962), which describes the dynamics of population size under density dependent conditions, demonstrates a so called chaotic behavior (May 1975). The generalized Ricker equations for pink salmon abundance are very sensitive even to extremely low levels of strays, which influence the long-term dynamics and the period of dominance shift (Zhivotovsky et al. 1995). In the same way, weak selection (even opposed by low levels of gene flow) can eventually result in large genetic divergence. The only difference between strong and weak forces is that the time needed for significant change is much longer for weak forces.

Gene drift also requires long times, of the order of N_e (Kimura 1983; Chakraborty and Leimar 1987), namely 10^5 to 10^7 generations for significant divergence among regional stocks as a result of random processes alone. The rate of change in gene differentiation, F_{ST}, under both gene flow and heterogeneous selection has not been analyzed. We can conjecture, however, that the rate is proportional to $1/m$ and $1/(s + t)$, so that if migration and selection are of the order of 10^{-3}, the number of generations is on the order of 10^3, much less than that for genetic drift.

Conclusion 3

In pink salmon, weak migration and weak heterogeneous selection may be important population forces and, therefore, cannot be neglected in interpreting gene differentiation. As a result, gene drift may play a role as a dynamic factor only at the lowest levels of hierarchy in small demes. At higher levels of pink salmon population hierarchy, gene drift may be overwhelmed even by weak migration and heterogeneous selection.

Another consideration is that each locus is acted on by different selection regimes. As a result, the frequencies of alleles at loci that are relatively neutral selectively may appear more homogeneous among local populations than those that are under local heterogeneous selection as a result of gene flow that is relatively unopposed by selection. Frequencies of alleles at other loci that are more strongly affected by heterogeneous selection will show more differentiation. Most of the data that have been accumulated for salmon is for allozymes, which may be relatively neutral. Genetic studies of life-history traits, however, many of which are polygenic, indicate the possibility of much stronger divergence than is reflected in allozyme data (Smoker et al. 1994).

Conclusion 4

Genetic data for characteristics other than biochemical genetic traits are required to more completely understand the hierarchical structure of salmonid populations. Such traits include, but are not limited to, quantitative morphological or physiological, behavioral, and adaptive traits such as fecundity, fertility, family size, temperature tolerance, and development rates as well as genotype–environment interactions.

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Genetic Comparison Between Pacific Herring (*Clupea pallasi*) and a Norwegian Fjord Stock of Atlantic Herring (*Clupea harengus*)

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Genetic studies on populations of Atlantic herring (*Clupea harengus* L.) in Norwegian seawaters have revealed a number of genetically distinct fjord populations. One population in Balsfjord in northern Norway was nearly fixed for several alleles that were very rare in the Atlanto-Scandian herring stock. A comparison with a sample of Pacific herring (*Clupea pallasi*) from British Columbia demonstrated that these alleles were identical to the more common alleles in this species. Genetic distance estimates based on six polymorphic loci demonstrated that Balsfjord herring were more similar to Pacific herring. Balsfjord and Pacific herring were also similar in vertebrae number and spawning behaviour. Restriction fragment analyses of mitochondrial DNA using five restriction enzymes revealed distinct clones that separated different herring groups. Nucleotide sequence divergence among groups was small.

Des études génétiques portant sur des populations de harengs de l'Atlantique (*Clupea harengus*) dans les eaux norvégienne (eau de mer) a révélé un certain nombre des populations génétiquement différentes dans les fjords. Une population dans Balsfjord, dans le nord de la Norgège, était presque fixe pour ce qui est de plusieurs allèles qui étaient très rares dans le stock de harengs atlanto-scandinave. Une comparaison avec un échantillon de hareng du Pacifique (*Clupea pallasi*) en provenance de Colombie-Britannique a montré que ces allèles étaient identiques aux allèles les plus fréquents dans cette espèce. L'estimation de la distance génétique fondée sur six loci polymorphes a indiqué que le hareng de Balsfjord ressemble davantage au hareng du Pacifique. Les harengs de Balsfjord et du Pacifique étaient également semblables pour ce qui du nombre de vertèbres et du comportement de frai. L'analyse des fragments de restriction de l'ADN mitochondrial réalisée à l'aide de cinq enzymes de restriction a révélé l'existence de clones distincts qui séparaient différents groupes de harengs. La divergence des séquences de nucléotides entre les groupes était faible.

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Population genetics studies have been carried out on Pacific herring, *Clupea pallasi* (Grant and Utter 1984; Schweigert and Withler 1990), and Atlantic herring, *Clupea harengus* (Kornfield et al. 1982; Ryman et al. 1984; Jørstad and Nævdal 1983; Grant 1986; King et al. 1987). In general, very low levels of genetic variation have been found within species in constrast to the variation observed in various life history characters.

Pacific and Atlantic herring have been considered to be two different subspecies, and Svetovikov (1963) recognized at least five different subspecies of Atlantic herring. Based on direct comparison of 40 allozyme loci between Atlantic and Pacific herring, Grant (1986) concluded that the two should be considered as separate species.

The low level of genetic variation in samples of Atlantic herring over large geographic areas (Kornfield et al. 1982; Grant 1984; Ryman et al. 1984; King et al. 1987) contrasted with genetic studies of herring in Norwegian waters. Evidence for genetically distinct fjord populations were obtained more than 10 yr ago, and analyses of 0-group herring from Balsfjord in northern Norway suggested the existence of several genetically differentiated herring stocks in these areas (Jørstad and Nævdal 1981).

The annual genetic screening program for herring in Norwegian waters (initiated in 1978) has revealed a number of genetically distinct populations of herring (Jørstad and Nævdal 1983). Of these, the Balsfjord herring appear to be the most differentiated genetically. Two different populations of herring (local Balsfjord and Atlanto-Scandian stock) can be found in this area, and these are almost fixed for different alleles at several polymorphic loci (Jørstad and Pedersen 1986). A comparison of results from genetic studies of other Atlantic herring (Jørstad et al. 1991), confirmed the genetic discreteness of Balsfjord herring.

Balsfjord herring were also characterized by a low vertebrae number and by intertidal spawning behaviour (Jørstad and Pedersen 1986), characteristics that suggest similarities with Pacific herring. The results from Grant's (1986) comparison between Pacific and Atlantic herring indicate an overlap of alleles at the two lactate dehydrogenase (*LDH*) loci expressed in white muscle. For this reason, it was of interest to carry out a direct genetic comparison of Balsfjord herring with Pacific herring. In this paper, results obtained by comparing samples of tissue enzymes on the same starch gel are presented. In addition, some preliminary results from mitochondrial DNA (mtDNA) analyses are given.

FIG. 1. Sampling areas of herring for genetic comparisons. (1) Ofotfjord = Atlanto-Scandian herring; (2) Balsfjord = Balsfjord herring; (3) British Columbia = Pacific herring.

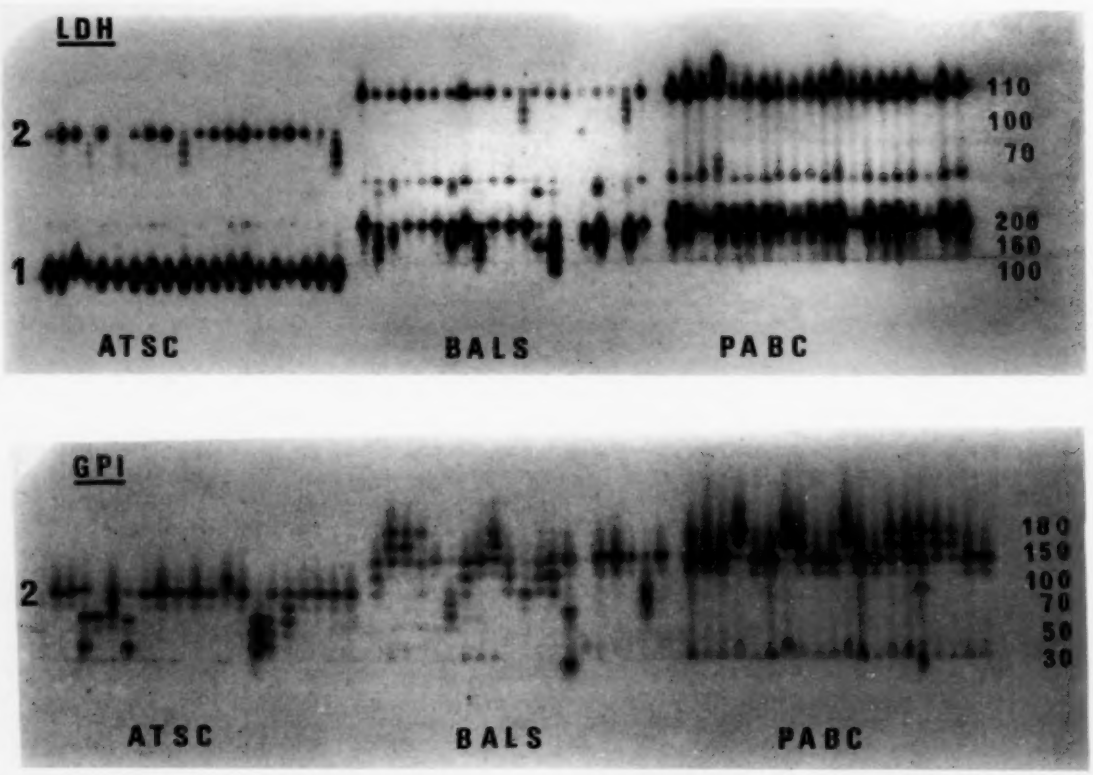

FIG. 2. Allozyme comparisons between Atlanto-Scandian herring (ATSC), Balsfjord herring (BALS), and Pacific herring from British Columbia (PABC) using starch gel electrophoresis and selective enzyme staining. The enzymes shown are lactate dehydrogenase (LDH) and glucosephosphate isomerase (GPI); the different loci (left) and alleles (right) are indicated.

Materials and Methods

A sample of Pacific herring from British Columbia was provided by Dr. Colin Levings of the Vancouver Marine Laboratory. Fish were caught in 1991 and kept frozen at −20°C before transportation to the Institute of Marine Research in Bergen, Norway, in April 1992. A fresh sample of Balsfjord herring was collected on the research vessel cruise, Michael Sars, in November 1991. At the same time, a sample of Atlanto-Scandian herring was caught in the overwintering area and used as a reference sample. All herring were stored at low temperature (−70°C) until analysis. Sampling localities are given in Fig. 1.

White muscle and liver were prepared from individual herring and analysed by horizontal starch gel electrophoresis (gel buffer: histidine, pH 7.0) and selective staining of enzymes (Harris and Hopkinson 1976). The following polymorphic enzymes were investigated: lactate dehydrogenase (*LDH-1**; *LDH-2**), isocitrate dehydrogenase (*IDHP-2**), maleate dehydrogenase (*MDH-4**), phosphoglucomutase (*PGM-1**) and glucosephosphate isomerase (*GPI-2**). These loci can be screened from white muscle. Allelic variation at each locus has been described elsewhere (Anderson et al. 1981; Kornfield et al. 1982; Grant 1981), and a comparison of alleles among different studies may be found in Jørstad et al. (1991). For genetic comparisons, the BIOSYS-1 program package of Swofford and Selander (1981) was used.

A limited number of individuals were examined for restriction length polymorphisms (RFLPs) of mtDNA. Gonads were dissected from 21 Pacific herring, six Balsfjord herring collected in 1989, and three Balsfjord herring collected in 1992.

MtDNA was extracted using the method described in Dahle and Eriksen (1990) and digested with several restriction enzymes. Only five enzymes (*Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, and *Sma*I) were used in final analyses. Digestion, electrophoresis, and fragment size estimations were carried out as described by Dahle and Eriksen (1990). As a reference sample for Atlanto-Scandian herring, 10 individuals were randomly chosen from the material presented in Dahle and Eriksen (1990). These individuals represented the most common mtDNA clones in the area.

Different mtDNA clones were identified by a five-letter code where each letter correspond to a specific fragment pattern for each restriction enzyme. Estimates of nucleotide sequence divergence were based on total fragment sizes and were calculated according to Upholdt (1987).

Results

Tissue samples from Balsfjord herring and Pacific herring were run on the same starch gel together with a reference sample of Atlanto-Scandian herring. Banding patterns of two of the most informative enzymes, LDH and GPI, are shown in Fig. 2. As observed previously (Jørstad and Nævdal 1983; Jørstad and Pedersen 1986), little variation was found at either *LDH* locus in Atlanto-Scandian herring. Balsfjord herring are almost fixed for the fast moving *LDH-2*120* allele, which is identical in electrophoretic mobility to the most common allele in Pacific herring (Fig. 2). The slow *LDH-2*70* allele has been observed only in Atlanto-Scandian herring where its frequency can be as high as 0.10 in some areas (Jørstad and Nævdal 1983).

A similar situation is found at the *LDH-1** locus. The fast *LDH-1*200* allele is not found in Atlanto-Scandian herring; however, the allele is found at high frequency in the Balsfjord herring and is fixed in Pacific herring.

TABLE 1. Comparisons of allele frequencies observed at six polymorphic loci in samples of Atlanto-Scandian herring, Balsfjord herring and Pacific herring from British Columbia. The number of alleles per locus and mean observed heterozygosity for the loci analyzed are also given.

Locus	Atlanto-Scandian	Balsfjord	Pacific
*LDH-1**			
(*N*)	60	58	90
**100*	0.983	0.052	0.000
**160*	0.017	0.259	0.000
**200*	0.000	0.690	1.000
*LDH-2**			
(*N*)	60	58	90
**70*	0.067	0.000	0.000
**100*	0.933	0.069	0.006
**120*	0.000	0.931	0.983
**127*	0.000	0.000	0.011
*MDH-4**			
(*N*)	60	58	90
**70*	0.483	0.155	0.039
**100*	0.517	0.845	0.961
*IDHP-2**			
(*N*)	60	58	90
**85*	0.000	0.052	0.000
**100*	0.992	0.112	0.394
**120*	0.008	0.836	0.594
**140*	0.000	0.000	0.011
*GPI-2**			
(*N*)	60	58	90
**30*	0.025	0.009	0.000
**50*	0.150	0.043	0.000
**70*	0.075	0.069	0.000
**100*	0.575	0.241	0.006
**150*	0.175	0.534	0.772
**180*	0.000	0.103	0.222
*PGM-1**			
(*N*)	60	58	90
**100*	0.983	0.526	0.822
**120*	0.017	0.474	0.178
No. of alleles per locus	2.50	3.0	2.33
(SD)	(0.50)	(0.63)	(0.33)
Mean observed heterozygosity	0.221	0.374	0.202
(SD)	(0.106)	(0.079)	(0.078)

Variation detected at the *GPI-2** locus is also shown in Fig. 2. Five different alleles were detected in Atlanto-Scandian herring, whereas six alleles were found in Balsfjord herring. Only three alleles, including one low-frequency allele, were found in Pacific herring. Comparison of allele frequency distributions among samples revealed that the *GPI-2*150* allele was at highest values in Balsfjord and in Pacific herring.

A summary of allele frequencies at six polymorphic loci for the three herring groups is given in Table 1. For all loci analyzed, there seem to be a similarity between the Balsfjord herring and the Pacific herring with respect to which allele at the different loci is occurring at the highest frequency. Not suprisingly, however, contingency chi-square tests (BIOSYS-1) between the two samples demonstrated highly significant allele frequency differences ($P < 0.01$) at all loci analysed. The highest P-value was estimated to 0.006 in

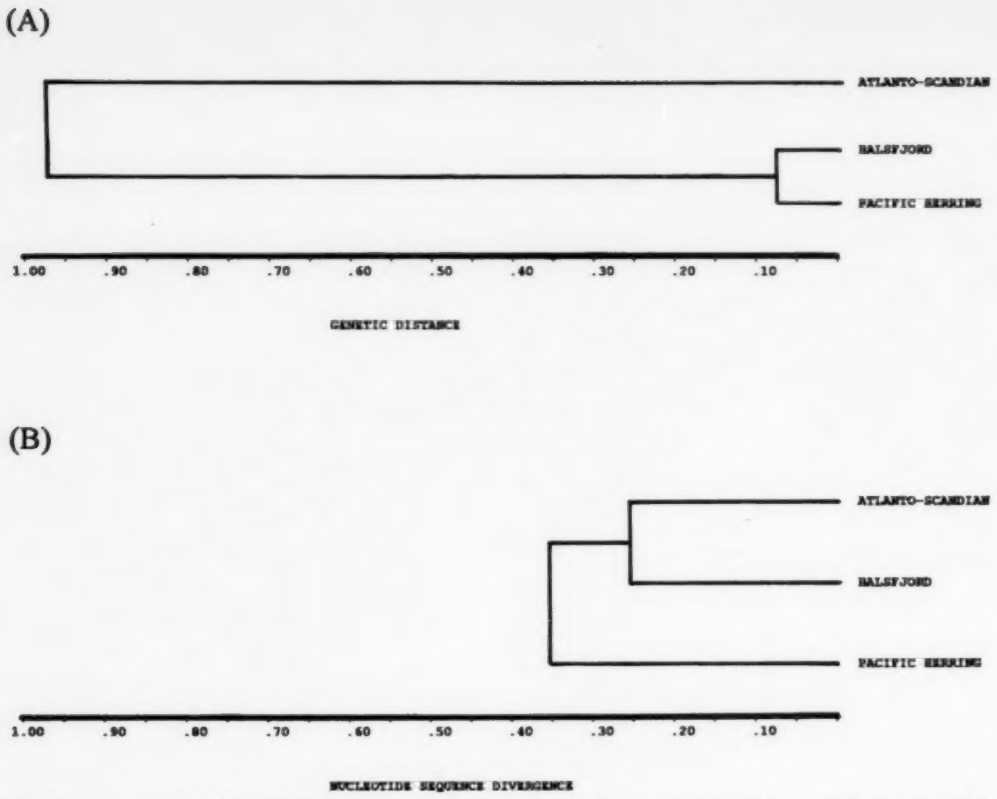

FIG. 3. Cluster dendrogram of (A) Nei (1978) unbiased genetic distance based on six polymorphic loci and (B) calculated nucleotide divergence (Upholdt 1987) between the different groups of herring.

TABLE 2. Distribution of mtDNA clones among the three different groups of herring. The order of restriction enzymes is *Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, and *Sma*I.

mtDNA clones	Atlanto-Scandian	Balsfjord	Pacific
AAAAA	5		
AAABA	1		
AABAA	1		
AACAA	1		
AAEAB	1		
ADAAA		1	
ADCEA		7	
DAAAA	1		
DAAEE			1
DDCEA		1	
EAAEE			6
EAAGE			1
EAAHE			1
EABAE			1
EABHE			5
EACHE			1
EAEEE			3
EBBEE			1
EADEE			1

the pairwise comparison at the *LDH-2** locus. Similar results were also obtained in contingency chi-square tests from all combinations of herring groups at all the loci in question.

The different samples were also tested for deviations from Hardy–Weinberg equilibrium expectations. No significant deviations from expected values were found at any of the loci examined. The number of alleles per locus was greatest Balsfjord herring (Table 1) and a significant lower value (2.33) were found in the Pacific herring. This was also expressed in the estimates of observed mean heterozygosity for the six loci, giving 0.374 for the herring in Balsfjord and 0.202 for Pacific herring.

Unbiased genetic distances of Nei (1978) were calculated based on allele frequencies at the six polymorphic loci (Table 1). The largest genetic distance (1.009) was found between Atlanto-Scandian and Pacific herring, whereas the lowest value (0.069) was between Balsfjord and Pacific herring. The genetic distance between Balsjord and Atlanto-Scandian herring was 0.926. In UPGMA cluster analysis, Balsfjord and Pacific herring constitute one branch of the dendrogram (Fig. 3) with a genetic distance of 0.967 to the Atlanto-Scandian herring.

Nineteen different mtDNA genotypes (clones) were found using five restriction enzymes (Table 2). No clones were shared by two populations. Six different clones were found in Atlanto-Scandian herring, and five individuals had identical restriction fragment patterns for the five enzymes. Three different clones were identified among Balsfjord herring and seven individuals had the same fragment patterns. Ten different clones were found among Pacific herring, and

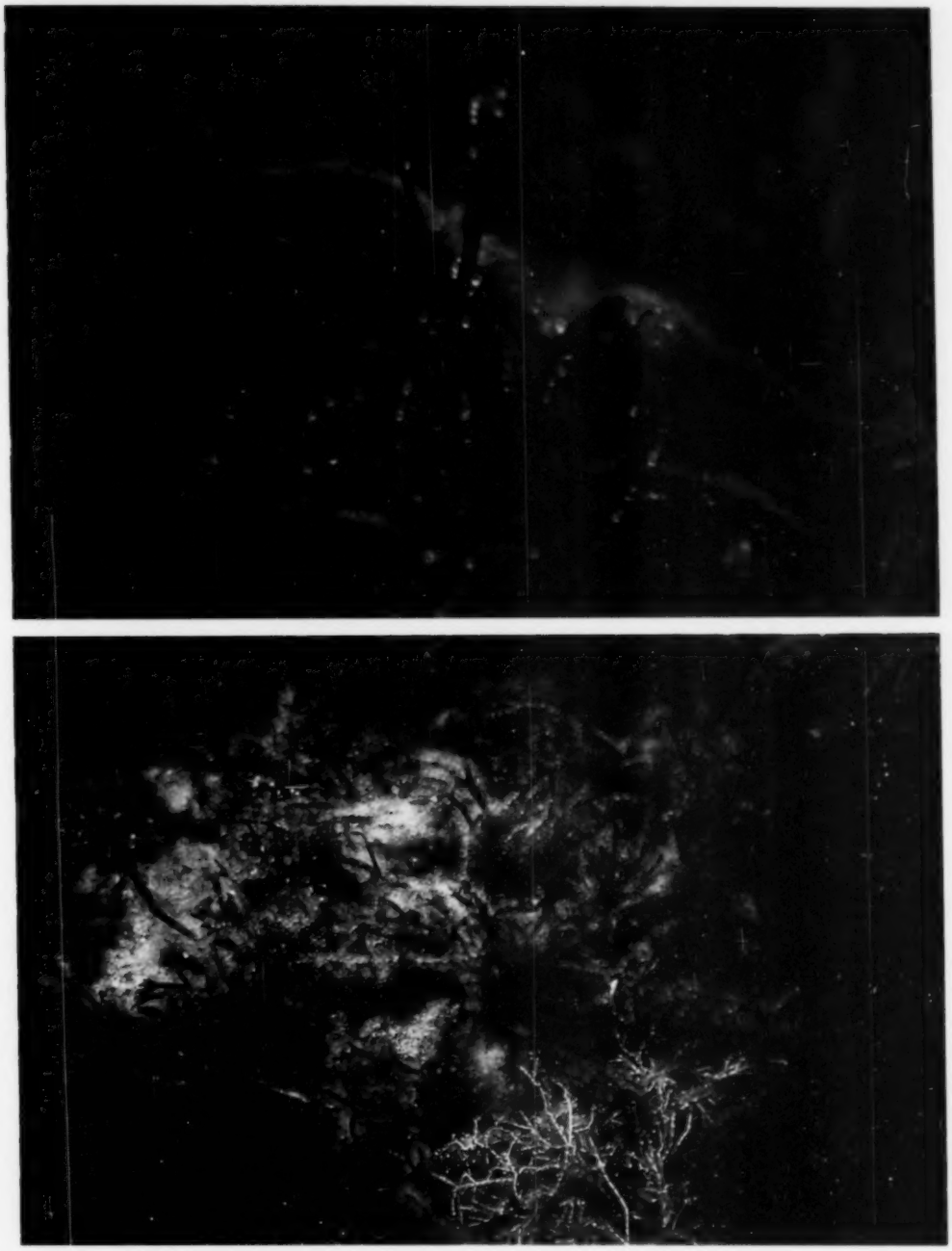

FIG. 4. Intertidal spawning of the Balsfjord herring at the innermost part of the fjord. Top: herring eggs exposed to air temperature at low tide. Bottom: underwater picture of the herring spawning bed (depth 1–2 m, high tide). (Photo: Eva Farestveit)

totally three of which were relatively common. Estimated nucleotide sequence diversity (Upholdt 1987) within the samples varied from 0.08 (Balsfjord) to 0.12 (both Atlanto-Scandian and Pacific).

Nucleotide sequence divergence within each group was significantly smaller than between the groups. In pairwise comparisons, the largest nucleotide sequence divergence (0.84) was between Pacific and Atlanto-Scandian herring, while the corresponding value between the latter and Balsfjord herring was 0.73. The value estimated between Balsfjord herring and Atlanto-Scandian herring was 0.54. A UPGMA dendrogram (Fig.3) indicated close similarity derived from nucleotide sequence divergence between Balsfjord herring and Atlanto-Scandian herring.

Discussion

Earlier studies (Jørstad and Nævdal 1983; Jørstad and Pedersen 1986) demonstrated significant variation (number of alleles and allele frequencies) at two *LDH* loci in herring. At the *LDH-2** locus, one rare allele (**120*) was nearly fixed in samples of Balsfjord herring. In contrast, no variation was reported for these loci in Pacific herring (Grant and Utter 1984; Schweigert and Withler 1990). In this study, the *LDH-2*120* allele found in Balsfjord herring was identical to the common allele at *LDH-2** in Pacific herring. A similar situation was seen at the *LDH-1** locus. The Atlanto-Scandian herring and Pacific herring were fixed for different alleles at both LDH loci. This confirms the results obtained earlier by Grant (1986).

Estimates of genetic distance presented here were based only on six polymorphic loci and cannot be compared with the results reported by Grant (1986). His study was based on 40 protein-coding loci and suggested that the two herring groups should be considered as separate species and not as subspecies (Svetovikov 1963). Our value of the genetic distance between Pacific herring and Atlanto-Scandian herring was 1.009 and was based on the most variable polymorphic proteins in herring. As such they are suited to study the genetic relationship between different herring groups and this is clearly demonstrated in the comparison between the Balsfjord herring and Pacific herring (genetic distance = 0.069).

Similarities between Balsfjord and Pacific herring are also indicated by other characters (Jørstad and Pedersen 1986). Included are the low number of vertebrae (Schweigert and Withler 1990) and intertidal spawning behaviour (Haegele and Schweigert 1985; Hay 1985). Jørstad and Pedersen (1986) found that the Balsfjord herring had a low number of vertebrae (range 54.1–54.8) compared to Atlanto-Scandian herring (range 57.1–58.0). With help from local people in the Balsfjord area, a specific spawning site for Balsfjord herring was localized at the innermost part of the fjord. At this site the local stock was spawning in April in the intertidal zone and deposited their eggs on the vegetation, mainly *Fucus* sp. and *Ascophyllum* sp. At low tide, parts of the vegetation and eggs are dry and exposed to air temperatures (Figs. 4A and 4B). In contrast, the main stock of Atlanto-Scandian herring spawns at 40–60 m depth on the coastal banks along the Norwegian coast (Parris and Saville 1967).

In the RFLP analyses, none of the nineteen identified mtDNA clones were found in two populations. Some enzymes appeared to have population specific patterns, e.g., pattern E of *Hin*dII and *Sma*I in the sample of Pacific herring. Kornfield and Bogdanowicz (1987) studied spawning groups of Atlantic herring in the Gulf of Maine and the Gulf of St. Lawrence using restriction enzyme analyses of mtDNA. They found no digestion pattern which could distinguish between different spawning groups, nor did restriction fragment studies of Atlantic herring from different localities in the North Sea detect substock structure (Dahle and Eriksen 1990). Nucleotide sequence divergence between the different groups of herring in this work were significanly larger than the values obtained for different localities in the North Sea (0.21–0.52; Dahle and Eriksen 1990).

These preliminary analyses of mtDNA include a limited number of individuals, especially with respect to the Balsfjord and the Atlanto-Scandian herring. For this reason additional material should be examined to determine if the difference in cluster analysis (Fig. 3) is valid.

The taxonomic status of Balsfjord herring is unclear. The similar characteristics of low vertebrae number and intertidal spawning behaviour between different subspecies of Pacific herring in the White Sea and adjacent waters (Svetovikov 1963) and Balsfjord herring suggest a possible relationship. The subspecies status of the herring in these area are mainly based on meristic and morphological characters. As discussed by Altukhov and Salmenkova (1987) qualitative differences between the populations have also been detected for genetic characters.

In constrast to limited genetic variation detected over large areas in the Pacific (Grant and Utter 1984; Schweigert and Withler 1990) and the different parts of the Atlantic (Kornfield et al. 1982; Ryman et al. 1984; Grant 1984; King et al. 1987), the genetic studies on herring in Norwegian waters have revealed variation both at the population (Jørstad and Nævdal 1983; Jørstad et al. 1991) and subspecies level (this study). The information available suggests that the northeast Atlantic, Barents Sea, and White Sea are areas where several distinct herring populations exist. Detailed knowledge of the genetic relationship among these groups are needed for fishery management and future conservation. In addition, this information is fundamental to understanding the evolution of the different herring species in the Pacific and Atlantic oceans.

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Phylogenetic Relationships among Palearctic and Nearctic Whitefish (*Coregonus* sp.) Populations as Revealed by Mitochondrial DNA Variation

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Bernatchez, L., and J.J. Dodson. 1994. Phylogenetic relationships among Palearctic and Nearctic whitefish (*Coregonus* sp.) populations as revealed by mitochondrial DNA variation. Can J. Fish. Aquat. Sci. 51(Suppl. 1): 240–251.

We recently documented the existence of two highly distinct mitochondrial DNA (mtDNA) phylogenetic groups of *Coregonus* sp. in Beringia in the absence of geographic separation. To test whether this resulted from secondary intergradation of two groups that evolved in allopatry in Eurasia and Beringia, mtDNA restriction-fragment-length polymorphisms of 210 whitefish representing 22 populations from Europe and Siberia were compared with mtDNA variation observed among 581 fish from North America. Results confirmed that Beringia represents a zone of secondary contact among endemic whitefish and a group of Eurasian origin. All Beringian populations clustered much more closely to Eurasian populations than other North American ones. We also compared mtDNA variation among Palearctic populations with that observed in North America. European populations clustered into two major mtDNA groups that exhibited a strong geographic pattern of distribution, independent of the morphological variation observed among populations: one dominated all the more northern populations and extended to Alaska–Yukon; the second largely dominated samples from central alpine lakes, and was absent from Beringia. These results suggest that central alpine lakes and northern Europe were postglacially recolonized by two genetically distinct whitefish groups that most likely evolved in allopatry followed by limited intergradation.

Nous avons récemment identifié deux groupes phylogénétiques de corégones (*Coregonus* sp.) en Béringie. Afin de vérifier l'hypothès voulant que le chevauchement de ceux-ci résulte d'un contact secondaire entre groupes allopatriques ancestraux, nous avons comparé la variation de l'ADN mitochondrial au sein de 210 corégones représentant 22 populations d'Eurasie à celle observée en Amérique du Nord. Les résultats ont confirmé l'hypothèse voulant que la Béringie représente une zone de contact secondaire entre un groupe de corégone endémique et un autre d'origine eurasienne. Toutes les populations béringiennes étaient plus apparentées aux populations européennes qu'à celles peuplant le reste de l'Amérique de Nord. D'autre part, les populations eurasiennes se divisaient en deux groupes génétiques démontrant une répartition géographique distincte, indépendamment de la variation morphologique. Un groupe dominait toutes les populations nordiques et se retrouvait jusqu'en Alaska et au Yukon. Le deuxième groupe dominait largement les populations lacustres des Alpes. L'ensemble de ces résultats suggère donc que les lacs alpins et l'Europe du nord ont été colonisés par deux groupes de corégones phylogénétiquement distincts qui ont évolué en allopatrie et entre lesquels le flux génique est demeuré restreint suivant leur dispersion postglaciaire.

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Pleistocene glaciation events are assumed to have dramatically altered the genetic population structure of northern fauna and flora (Denton and Hughes 1981). Alternate phases of glacial advances and retreats may have been as important in promoting radiation of local populations as in favoring intermittent gene flow among them through their subsequent dispersal (Briggs 1986). Thus, during glacial advances, several races may have been created by temporal and geographical isolation in separate glacial refugia. Conversely, the opening of dispersal routes has provided opportunities for gene flow among newly emerging races.

Northern freshwater fish species remained geographically isolated in separate drainages during the maxima of Pleistocene glaciations. However, these geographically isolated populations were intermittently provided with opportunities for widespread dispersal. Thus, major continental dispersal routes became available during phases of deglaciation through the creation of large-scale hydrographic connections and development of proglacial lakes associated with glacier melting (Fulton and Andrews 1987). Opportunities for intercontinental dispersal of freshwater fishes were also created during phases of glacial advance by the episodic openings of the Bering land bridge, classically recognized as the major route for faunal exchange between Eurasia and North America (Kontrimavichus 1973; Lindsey and McPhail 1986).

The whitefishes are members of the family Salmonidae and have a continuous circumpolar distribution in the northern hemisphere. The Palearctic and Nearctic populations of whitefish have classically been divided into two major nomenclatural species complexes based on continental distribution;

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the European whitefish (*Coregonus lavaretus*) and the lake whitefish (*Coregonus clupeaformis*). The wealth of phenotypic variation observed in whitefish had led to various taxonomic interpretations of numerous geographical and ecological forms within each species complex (Berg 1948). This situation had led to a very confusing and inconsistent nomenclature that still prevails in the literature today (e.g., Lelek 1987; Page and Burr 1991). Conversely, it has been proposed that whitefish should be considered as a single species because of low genetic differentiation detected in allozyme studies, and absence of either demonstrated reproductive isolation, or diagnostic morphological and allozyme characteristics between forms (Bodaly et al. 1991).

Whitefish are a typical component of northern freshwater communities and support important fisheries in most northern countries. Coregonines display a phenotypic plasticity that far exceeds what is usual in the animal kingdom (Mayr 1963), and this has hampered the understanding of their evolutionary history for over a century (Lindsey 1988). A major point of confusion in the understanding of the evolutionary history of whitefish concerns the phylogenetic relationships between Eurasian and North American populations and the role that Beringia (the region encompassing Yukon, Alaska, and the most eastern part of Siberia) has played in connecting and/or isolating the two groups during the Pleistocene.

Several studies have documented the genetic structure of whitefish on each continent separately (Franzin and Clayton 1977; Heinonen 1988; Vuorinen et al. 1986, 1993; Bodaly et al 1992). Recently, Bodaly et al. (1991) suggested, on the basis of allozyme variation, that Beringian whitefish may be more closely related to European populations than to other North American ones. However, there has been no attempt to address specifically the historical biogeography of these populations.

In recent years, analysis of the spatial distribution of polymorphisms in the nucleotide sequence of mitochondrial DNA (mtDNA) has been effective in studies of historical biogeography (Avise 1992) and particularly in demonstrating the influence of Pleistocene climatic fluctuations in shaping intraspecific genetic structure (e.g., Bernatchez et al. 1989; Lamb et al. 1989; Martin and Simon 1990; Riddle and Honeycutt 1990; Bernatchez et al. 1992; Riddle et al. 1993). In a study of the mtDNA phylogeographic structure of North American whitefish (Bernatchez and Dodson 1991), we observed four distinct phylogenetic groupings that were associated with four whitefish glacial races. A major phylogenetic break was observed between Beringia and the rest of North America, which corroborated previously documented discontinuities in morphology and isozyme frequencies (Franzin and Clayton 1977). In contrast to previous allozyme data, our study revealed the existence of two phylogenetic groups from Beringia that were as divergent from each other as they were from disjunct phylogenetic groupings characteristic of all other North American lake whitefish. The distribution of these two mtDNA groups overlapped among Beringian populations. As a scenario of stochastic lineage sorting appeared unlikely to account for this observation (Bernatchez and Dodson 1991), we proposed that this pattern of high phylogenetic discontinuity in the absence of geographic separation may have resulted from the secondary intergradation of two whitefish groups that evolved allopatrically in Eurasia and Beringia (Alaska–Yukon). In the light of those results, we extended our mtDNA analysis of whitefish populations to Europe and western Siberia to satisfy two complementary objectives: (1) to verify whether the closer genetic relationships between Eurasian and Beringian whitefish populations compared with other North American ones documented from allozyme data was also reflected in the pattern of mtDNA variation, and (2) by combining our results obtained previously for North America with those reported here for Eurasia, to present a comparison of mtDNA variation between Palearctic and Nearctic whitefish populations to propose a biogeographical hypothesis explaining the pattern of genetic differentiation observed among contemporary whitefish populations worldwide.

Materials and Methods

Sample Collection and Restriction Enzyme Analysis

A total of 210 Eurasian whitefish were collected from 1987 to 1990 among 22 populations, in addition to those 581 whitefish representing 41 populations from Beringia and the rest of North America previously reported in Bernatchez and Dodson (1991) and Vuorinen et al. (1993) (Table 1, Fig. 1). Twelve whitefish were sampled on average at each locality. Most specimens were adult fish captured during their spawning run. Fresh or frozen egg and liver samples were shipped by collaborators or transported by ourselves to the laboratory.

MtDNA was purified from liver and egg tissues by the rapid extraction method of Chapman and Powers (1984) as modified by Bernatchez et al. (1988). MtDNA aliquots were digested with eight hexameric (*Dra*I, *Hin*dIII, *Pvu*II, *Sma*I, *Xmn*I, *Bgl*I, *Pst*I, *Bam*HI), four multihexameric (*Ava*I, *Ban*I, *Hae*II, *Hin*cII) and one multipentameric (*Ava*II) restriction enzymes. MtDNA fragments were electrophoretically separated on 0.8% and 1.2% agarose gels. Restriction fragments were revealed either by ethidium bromide staining or hybridization with a highly purified radiolabelled total mtDNA probe (Bernatchez and Dodson 1990a). Fragment sizes were estimated by comparison with digests of phage λ DNA with *Hin*dIII and *Eco*RI–*Hin*dIII double digest. No attempt to visualize fragments less than 350 base pairs was made. Distinct single endonuclease patterns were identified by a specific letter in order of appearance. Each fish was assigned a multiletter code which described its mtDNA haplotype.

Data Analysis

The 53 mtDNA genotypes identified in Eurasian populations were added to the 44 described by Bernatchez and Dodson (1991). The entire data set was subjected to both distance and character-based analyses. The distance measures used were estimates of nucleotide sequence divergence (P) among mtDNA genotypes calculated by the site approach of Nei and Li (1979). Using the same method that was used for North American whitefish (Bernatchez and Dodson 1991), site differences between Eurasian whitefish mtDNA genotypes were inferred from changes in fragment patterns because these could be accounted for by specific site gains or losses. The resulting restriction site presence–absence matrix was used in character-based analyses to generate phylogenetic trees according to Wagner parsimony criteria using the MIX program from the PHYLIP 3.4

TABLE 1. Location of sampled whitefish (*Coregonus* sp.) populations. Numbers preceding location names refer to positions on the map. Taxonomic designations refer to traditional morphological classification largely based on continent of origin and gill-raker numbers and still widely used at the present time (sources: Scott and Crossman 1973; Lelek 1987; Page and Burr 1991).

Sample location	Traditional taxonomic designation	*N*
1. Mira R., Nova Scotia	*clupeaformis*	12
2. Grand L., New Brunswick	*clupeaformis*	12
3. L. Témiscouata, Québec	*clupeaformis*	12
4. Cliff L., dwarf ecotype, Maine	*clupeaformis*	16
5. Spider L., dwarf ecotype, Maine	*clupeaformis*	12
6. Second Musquacook L., Maine	*clupeaformis*	9
7. Cliff L., normal ecotype, Maine	*clupeaformis*	12
8. L. Saint-François, Québec	*clupeaformis*	12
9. L. Champlain, Québec	*clupeaformis*	12
10. Saint-Lawrence R., Québec	*clupeaformis*	13
11. Réservoir Kipawa, Québec	*clupeaformis*	12
12. Koksoak R., Québec	*clupeaformis*	15
13. Povungnituk R., Québec	*clupeaformis*	24
14. Réservoir Manic V, Québec	*clupeaformis*	10
15. Squaw L., Québec	*clupeaformis*	12
16. Altikamagen L., Québec	*clupeaformis*	9
17. Inukjuak R., Québec	*clupeaformis*	12
18. Great Whale R., Québec	*clupeaformis*	9
19. La Grande R., Québec	*clupeaformis*	20
20. Eastmain R., Québec	*clupeaformis*	31
21. Rupert River, Québec	*clupeaformis*	15
22. L. Ontario, Ontario	*clupeaformis*	12
23. L. Huron, Ontario	*clupeaformis*	12
24. Como L., dwarf ecotype, Ontario	*clupeaformis*	36
25. Como L., normal ecotype, Ontario	*clupeaformis*	44
26. L. Superior, Ontario	*clupeaformis*	10
27. L. Michigan, Michigan	*clupeaformis*	11
28. L. Michigan, Michigan	*clupeaformis*	11
29. South Indian L., Manitoba	*clupeaformis*	12
30. Jack Fish L., Sakatchewan	*clupeaformis*	12
31. Wabamum L., Alberta	*clupeaformis*	12
32. Great Slave L., Northwest Territory	*clupeaformis*	12
33. Crooked R., British Columbia	*clupeaformis*	12
34. Fort McPherson, Northwest Territory	*clupeaformis*	12
35. Arctic Red R., Northwest Territory	*clupeaformis*	14
36. Squanga L., low gill-raker ecotype, Yukon	*clupeaformis*	12
37. McEvoy L., Yukon	*clupeaformis*	12
38. Dezadeash L., low raker ecotype, Yukon	*clupeaformis*	9
39. Chatanika R., Alaska	*nelsoni*	12
40. Yukon R., Alaska	*nelsoni*	11
41. Minnesota L., Alaska	*pidschian*	12
42. Kiiminkijoki R., Finland	*lavaretus*	14
43. Mickelsörarna, Finland	*widegreni*	20
44. Öja, Finland	*lavaretus*	12
45. Kyrönjoki R., Finland	*lavaretus*	8
46. Lestijoki R., Finland	*lavaretus*	11
47. Malaxa R., Finland	*lavaretus*	4
48. Dyhajarvi L., Finland	*muksun*	4
49. Kymijoki R., Finland	*lavaretus*	6
50. Niewleno L., Poland	*lavaretus*	11
51. Sperillen L., Norway	*lavaretus*	11
52. Constance L., Blaufelchen, Switzerland	*wartmani*	12
53. Constance L., Gangfisch, Switzerland	*oxyrhynchus*	12
54. Brienz L., winter spawning, Switzerland	*oxyrhynchus*	12
55. Brienz L., spring spawning, Switzerland	*oxyrhynchus*	12
56. Brienz L., fall spawning, Switzerland	*oxyrhynchus*	12
57. L. Léman, France/Switzerland	*fera = nasus*	10
58. L. d'Aiguebelette, France	*lavaretus*	12
59. L. d'Annecy, France	*lavaretus*	5

TABLE 1. (*Concluded*)

Sample location	Traditional taxonomic designation	*N*
60. Loch Lomond, Scotland	*lavaretus*	8
61. Kola Peninsula, Russia	*lavaretus*	1
62. Lapin R, Siberia	*pidschian*	2
63. Sos'va R., Siberia	*pidschian*	2

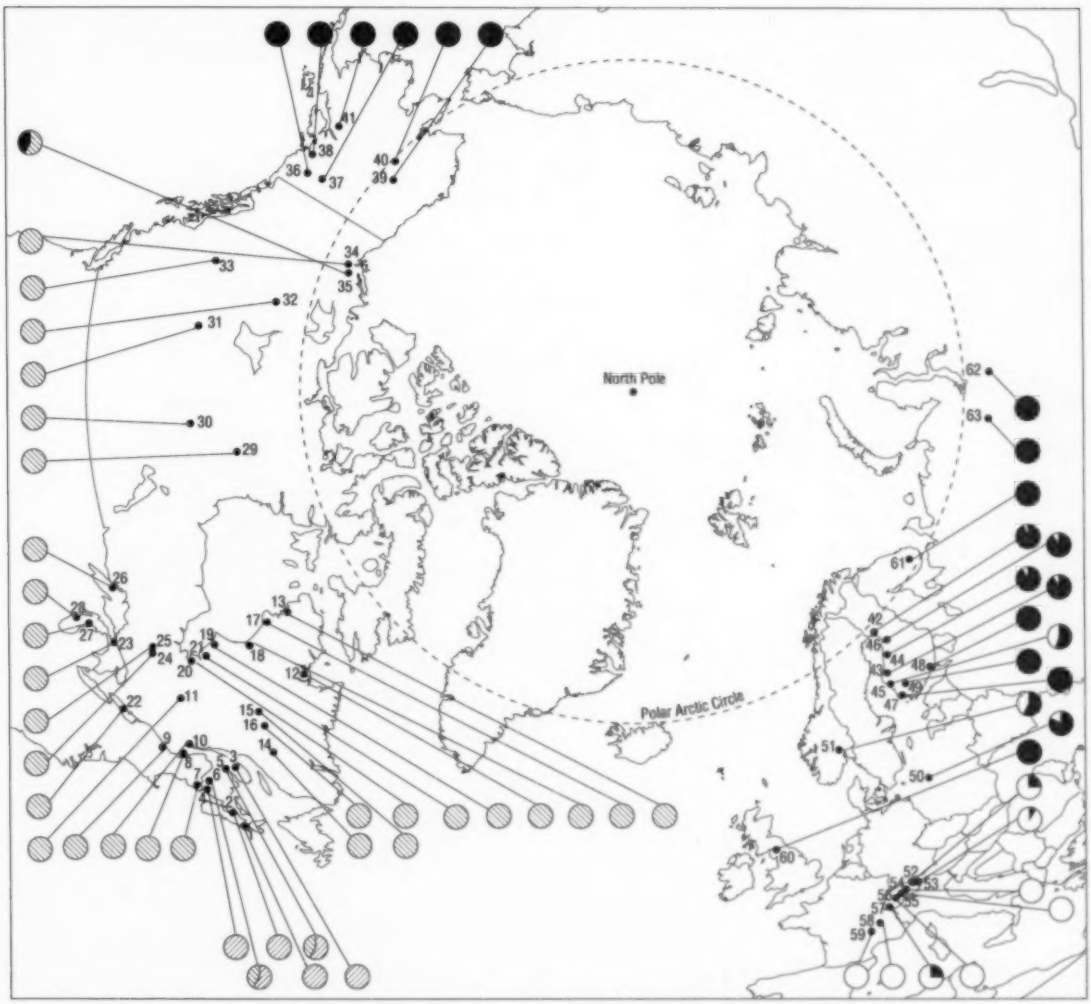

FIG. 1. Location map of sampled whitefish populations, and geographic distribution of major mtDNA phylogenetic groups identified in character-based analysis. Group I = cross hatched; group Ia = reversed cross-hatched; group II = dotted; group III = solid; group IV = open.

computer package (Felsenstein 1992). Synplesiomorphic and autapomorphic characters were omitted from the site matrix, leaving only phylogenetically informative data for tree construction. As numerous trees of equal length were found, a consensus tree and bootstraping estimates on branches were estimated by running 100 bootstrap replicates with the BOOT program from PHYLIP. Phylogenetic trees were rooted to a close relative of whitefish, the vendace (*C. albula*) (Bodaly et al. 1991; Bernatchez et al. 1991). As a measure of inter- and intra-population genetic diversity, we also computed the maximum-likelihood estimation of the average number of nucleotide substitutions per site within populations (π, nucleotide diversity) and among populations (d, nucleotide divergence) (Nei and Tajima 1981, 1983; Nei 1987). The

TABLE 2. Fragment size estimates (in base pairs) of additional fragment patterns observed among Eurasian whitefish populations and that were not described in Bernatchez and Dodson (1991). Fragments marked with asterisks were not observed but assumed under the criteria of minimum mutational steps involved in fragment changes. Enzymes *Bam*HI, *Pvu*II, and *Pst*I revealed no additional fragment patterns.

*Ava*I	G	H	I	J	K	L	M	N	O	P
3800		—		—						
3700	—	—		—			—	—	—	
3450				—						
3250		—								
2800						—				—
2500	—	—	—	—	—	—	—	—	—	—
2500									—	—
2400	—				—	—				
2300			—					—		
1950	—	—	—	—	—	—	—	—	—	—
1650							—			
1500	—	—	—	—	—	—		—	—	—
1450	—	—	—	—	—	—	—		—	—
1250	—	—	—	—	—	—	—	—	—	—
1150	—	—	—		—	—	—	—	—	—
900						—				—
850	—		—	—	—	—	—	—	—	—
800								—		
640								—		
*200	—	—	—	—	—	—		—	—	—
*100	—	—		—	—	—	—	—	—	—
*100	—	—	—	—	—	—	—	—		
*100			—	—				—		

*Ava*II	M	N	O	P	Q	R	S	T
3400	—		—	—	—	—		—
3050		—						
2750	—	—	—	—	—	—	—	—
2300							—	—
2270			—					
1475	—	—	—	—	—	—	—	—
1475					—			
1450	—	—	—	—	—	—	—	—
1300	—	—		—				—
1280							—	
1050						—	—	—
970	—	—		—	—		—	—
870				—				
830		—						
710	—	—	—	—	—	—	—	—
680	—	—	—	—	—	—	—	—
560	—	—						
550	—		—	—	—	—	—	
550	—	—	—	—	—	—	—	—
500			—		—	—	—	—
470	—	—	—	—	—	—	—	—
470	—	—	—	—	—	—	—	—
360		—						
350	—	—	—	—	—	—	—	—
350	—		—		—	—	—	—
*250			—		—	—	—	
*200	—	—	—	—		—	—	—
*150	—	—	—	—	—	—	—	—

*Ban*I	L	M	N
4900	—	—	
3700	—		—
3250			—
2700			
2500		—	
2200			
2000	—	—	—
2000		—	
1700	—		—
1650	—	—	—
1650			—
1200		—	
1150	—	—	—
670	—	—	—
*300	—		—
*Sma*I			
		D	
5550	—		
5550	—		
3600	—		
2000	—		
*200	—		

*Bgl*I	C	
9400	—	
5800	—	
1800	—	
2700		
2500	—	
*Xmn*I	E	
2200		
2000	—	—
7600	—	
5050	—	
2850	—	
1650		—
685	—	
1200	—	
630	—	
1150	—	—
670	—	—
*Dra*I		
	B	C
6000	—	
5560		—
4600		—
4200	—	
3300	—	—
2275	—	—
630	—	—
440		—
400	—	

*Hind*III	H	I	J	K
3750	—		—	—
3750			—	
3500		—	—	—
3500	—	—		
3200	—	—	—	—
3000		—		
2880	—			
2700				—
2400	—	—	—	—
800				—
750		—		
620	—			
470	—	—	—	—
*250	—	—		—
*Hae*II				
	J	K	L	
5600	—			
5150		—	—	
4800	—	—	—	
2800	—	—	—	
1640		—		
1400	—	—	—	
1000			—	
940	—	—	—	
940	—			
700	—		—	
440		—	—	

*Hinc*II	G	H	I	J	K	L	M	N	O	P
5550	—						—			
5200						—				
4250		—	—	—		—		—	—	—
4100				—		—				
4000		—							—	
3900								—		
3250				—						
2350	—		—		—		—			—
2250	—	—	—		—	—	—	—	—	—
2000									—	—
1800				—						
1675			—				—			—
1575	—									
1300		—	—	—					—	—
1250								—		
1125	—	—	—	—	—	—	—	—	—	—
1125	—	—	—	—	—	—	—	—	—	—
1075	—	—	—		—	—	—	—		
930	—	—	—	—	—	—	—	—		
810	—	—	—	—	—	—	—	—	—	—
*100	—	—	—			—	—	—	—	—
*100	—						—			
*50							—			

distance matrix of net nucleotide divergence among populations was used to construct a phenogram clustering all Nearctic and Paleartic populations by UPGMA.

Results

Phylogenetic Differentiation of mtDNA Groupings and their Geographic Distribution

The 13 enzymes used allowed the recognition of 138 different restriction sites that comprised a total of 45 synapomorphic sites. Fragment patterns generated for each enzyme among North American populations are detailed in Bernatchez and Dodson (1991), whereas additional patterns observed only among Eurasian populations are presented in Table 2. A total of 97 differentiated whitefish mtDNA haplotypes were observed among all 63 populations. A description of composite haplotypes observed in North America along with their distribution was presented by Bernatchez and Dodson (1991), and those observed in Eurasia are presented in Table 3. As illustrated by the topology of the majority-rule consensus tree (Fig. 2), all haplotypes clustered in five major mtDNA groups that were supported at more than 75% bootstrap level, indicating that they represented five distinct monophyletic groupings. The rooting of the tree with *C. albula* was between groups I–II and groups III–IV assemblages.

MtDNA genotypes observed in Beringia belonged to two distinct phylogenetic groups (Fig. 1). Group II consisted of mtDNA genotypes endemic to Beringia and corresponds to phylogenetic group A of Bernatchez and Dodson (1991). MtDNA genotypes that composed the second Beringian group (B in Bernatchez and Dodson 1991) clustered with numerous additional genotypes (group III) that were widely distributed among European and Siberian populations (Fig. 1). This mtDNA group dominated all Beringian populations sampled. Mean percent sequence divergence among Beringian and Eurasian mtDNA haplotypes falling within this same phylogenetic group B generally differed by less than $P = 0.005$ (range 0.001–0.0057) and generally implied few mutational changes. For instance, the most abundant and widespread haplotype observed in Beringia (haplotype 29), differed by a single site ($P = 0.001$) from one of the most abundant genotypes (genotype 92) found in Finland and Siberia, two restriction sites ($P = 0.002$) from genotype 97 found in Finland, and two restriction sites ($P = 0.002$) from genotype 66 observed in Europe.

In addition to mtDNA group III, haplotypes observed among European populations clustered in an additional major mtDNA group (IV) differing by a *Xmn*I and a *Ban*I diagnostic restriction site (Fig. 2). Phylogenetic groups III and IV exhibited a strong geographic pattern of distribution (Fig. 1). Group III dominated all the more northern populations from Scotland, Norway, Finland, and Russia, as well as the Polish population, representing 92% of all whitefish from these locations. In fact, group III was also fixed in three populations and dominated in all others from Alaska and Yukon, representing 78% of all whitefish sampled. Conversely, group IV characterized 88% of all whitefish samples from central alpine lakes in France, Switzerland, and Germany, and was absent from Beringia. In contrast, extensive dispersal of several individual mtDNA haplotypes was observed in very distant populations belonging to well-isolated water drainages (Table 1). For instance, genotype 66 was observed in Poland, Switzerland, Finland, Norway, and Scotland, and genotype 87 was observed throughout the European range except in Scotland. It is, however, not possible to refute the possibility that the apparent dispersal is the result of artificial stock transfer from one region to the other.

All whitefish haplotypes sampled in North America outside Beringia constituted a mtDNA group (group I) that showed a distinct phylogeographic separation from other groups (Fig. 1). This assemblage corresponds to phylogenetic group C described in Bernatchez and Dodson (1991). Within this group, haplotypes 25–28 (Group Ia) also formed a distinct assemblage (group D in Bernatchez and Dodson 1991) that exhibited a disjunct geographic distribution. This assemblage characterized whitefish of Acadian refugium origin (Bernatchez and Dodson 1991). Distinct geographic patterns of distribution exhibited by other minor haplotype groupings (haplotypes 17–22) suggested the existence of whitefish issued from an Atlantic refugium (detailed in Bernatchez and Dodson 1991).

Nucleotide Diversity Among and Within Populations

The distance matrix of net nucleotide divergence among populations was used to construct a phenogram clustering all studied populations (Fig. 3). The tree clearly showed that Beringian populations clustered more closely to European–Siberian ($d < 0.003$) than other North American populations ($d = 0.009$). This reflects the dominance of phylogenetic group III in Beringia. However, Beringian populations did not compose a distinct cluster and were also highly differentiated among themselves, in some instances as much as they were from European populations. This was largely caused by the differential distribution of phylogenetic groups II and III as well as fixation of unique haplotypes in some populations (e.g., Dezadeash Lake and Minnesota Lake; Table 1). In Europe and Siberia, nucleotide divergence among populations was very low in all cases ($d < 0.002$). Nevertheless, two major population clusters were observed. Populations of central alpine lakes clustered distinctively from all others, which reflects the predominance of mtDNA Group IV in this region. The other population cluster included all populations from England, Scandinavia, Russia, and Poland. The interpopulation genetic differentiation within each cluster was extremely low, as demonstrated by d values that were generally less than 0.0005. Within North American populations outside Beringia, those that composed the Acadian assemblage described by Bernatchez and Dodson (1991) also composed a distinct cluster.

No clear association was observed between population clustering patterns and traditional taxonomic designation. For instance, populations classified as *C. clupeaformis* belonged either to the distinct North American or Beringian–Eurasian groupings (Table 1, Fig. 3). Conversely, populations with different taxonomic designation fell within the same cluster and were very slightly genetically differentiated (e.g., *C. lavaretus, C. oxyrhynchus,* and *C. fera* in central Europe cluster or *C. lavaretus, C. wartmani, C. muksun,* and *C. pidschian* in the northern Europe cluster).

Figure 3 illustrates the distinction in nucleotide diversity observed within non-Beringian North American populations as opposed to Beringian and European populations. Nucleotide diversity in North America was nil or extremely low in most cases (mean = 0.0007), with the exception of the Mackenzie Delta population, which represented the only

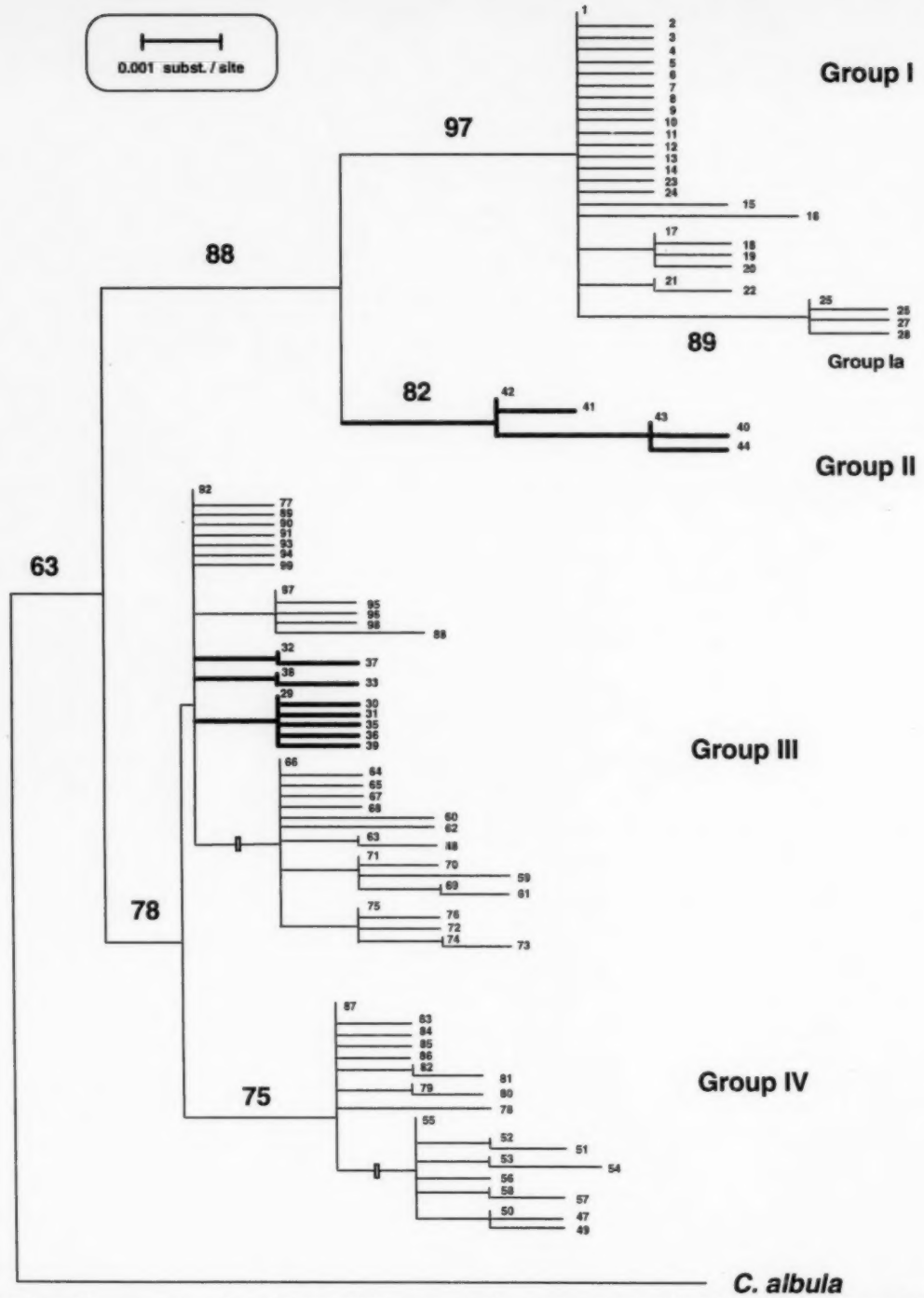

FIG. 2. Majority-rule consensus tree clustering 97 mtDNA genotypes observed among 791 whitefish representing 63 populations. Numbers at the termini refer to haplotype definitions in Table 1. Group I, Ia, II, and III correspond to phylogenetic groups C, D, A, and B, respectively, described in Bernatchez and Dodson (1991). Branches in bold identify haplotypes that were found in Beringia only. Bootstrap estimates (in percentages) are given along branches. The tree was rooted using *C. albula* as an outgroup. The scale of 0.001 substitution/site corresponds to one restriction site difference. The small rectangles across branches in Group III and IV indicate length mutations.

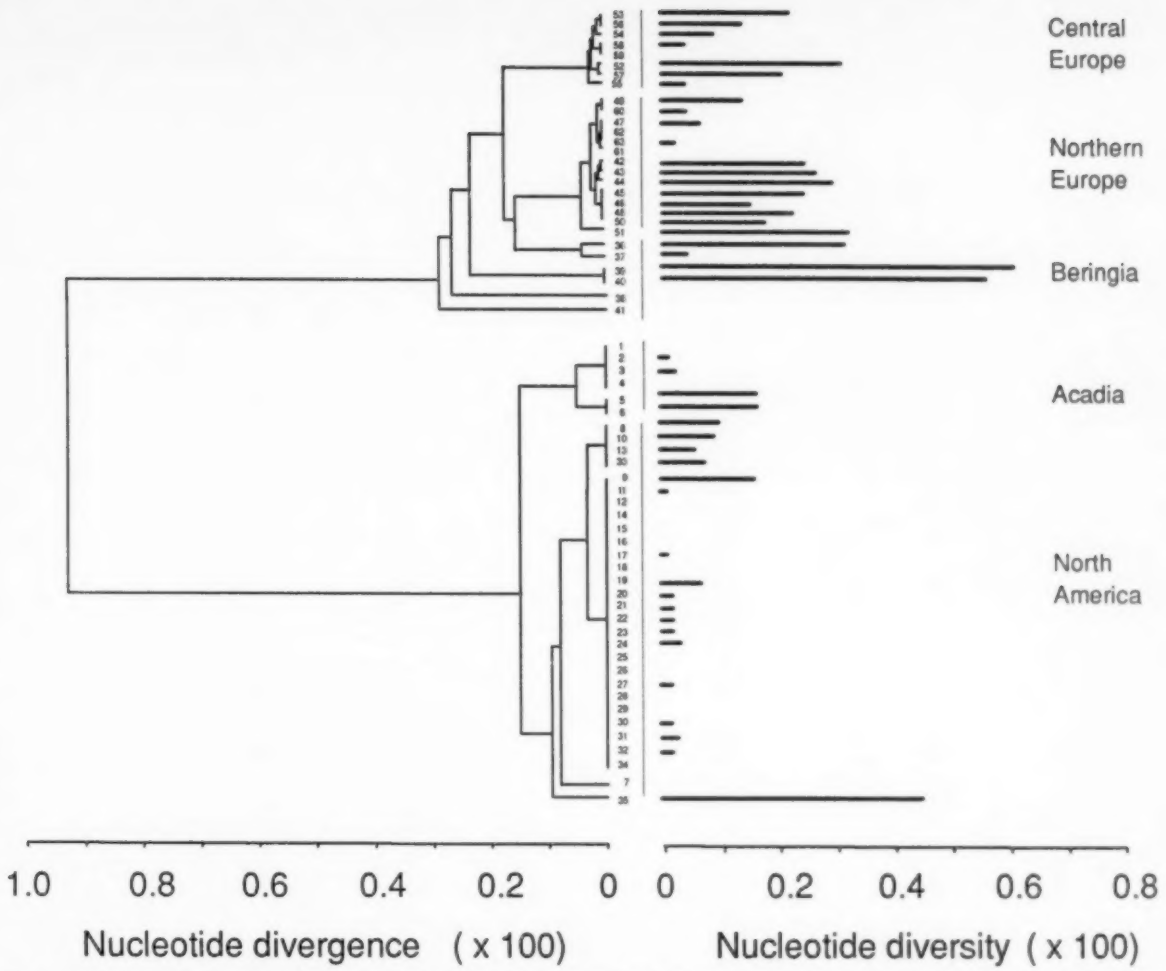

FIG. 3. UPGMA phenogram clustering whitefish populations according to the distance matrix resulting from maximum likelihood estimation of the net average number of nucleotide substitutions per site between populations (d, nucleotide divergence; Nei 1987). Intrapopulation nucleotide diversity (π) is expressed graphically on a horizontal scale next to each population. Numbers next to each population refer to Fig. 1. Names of the five major population clusters refer to their broad geographic distribution: Acadia (populations of Eastern North America diagnostically characterized by group Ia); North America (populations characterized by phylogenetic group I (excluding Ia)); Beringia (includes populations from Alaska and Yukon); central Europe (refers to central alpine lakes populations); and northern Europe (refers to Scottish, Scandinavian, Polish, and Russian populations).

overlap of Beringian (group III) and other North American (group I) haplotypes. In contrast, Beringian and European populations typically exhibited higher nucleotide diversity (mean = 0.0026, and 0.0018, respectively).

Discussion

Phylogeographic Patterns among Palearctic and Nearctic Whitefish Populations

It was conceivable, a priori, that divergent mtDNA groups were by chance retained within the same Beringian whitefish population in the absence of historical population separation (Neigel and Avise 1986). However, this scenario appears unlikely for the following reasons. First, such a stochastic model of lineage sorting cannot account for the nonstochastic distribution of the two Beringian mtDNA groups observed in Eurasia. Second, nonstochastic spatial discontinuity among much less differentiated mtDNA clades was observed in other regions of the whitefish distribution range (Bernatchez and Dodson 1990b). Third, although the retention of divergent (approximately 1% divergence and more) lineages within the same population is theoretically possible if long-term effective population size remains large, there is very little empirical support for that scenario in the literature, as this phenomenon has been observed in only four instances in vertebrates (Avise et al. 1992).

Therefore, the most rational explanation for the geographic distribution of these groupings appears to be that Beringia was recolonized by a whitefish group that dispersed extensively in Eurasia, and subsequently intergraded with an endemic Beringian whitefish group. Also, the much closer genetic relationships between Eurasian and Beringian whitefish populations

TABLE 3. Composite definitions, absolute abundance (*N*), and geographic localization of whitefish mtDNA haplotypes observed among 22 Palearctic whitefish populations. Haplotype numbers start from 47 for a direct continuation of Nearctic whitefish haplotypes described in Table 2 of Bernatchez and Dodson (1991). Restriction enzymes are (in order) *Aval*, *Ava*II, *Ban*I, *Bgl*I, *Dra*I, *Hae*II, *Hin*cII, *Hin*dIII, *Pvu*II, *Sma*I, *Xmn*I, *Bam*HI, and *Pst*I. Capital letters refer to fragment patterns described in Bernatchez and Dodson (1991) and in Table 2. Population numbers refer to location names listed in Table 1.

	Composite haplotypes													*N*	Population
47	O	L	L	B	A	L	J	J	B	A	B	A	A	1	55
48	P	L	G	B	A	L	L	J	B	A	A	A	A	1	52
49	O	M	L	B	A	I	K	J	B	A	B	A	A	1	55
50	O	M	L	B	A	L	L	J	B	A	B	A	A	1	55
51	G	L	N	B	A	I	H	G	B	A	B	A	A	2	52,53
52	G	L	L	B	A	I	H	G	B	A	B	A	A	1	52
53	G	M	L	B	A	I	O	G	B	A	B	A	A	2	52,53
54	M	M	G	B	A	I	P	G	B	A	B	A	A	2	52,53
55	G	M	L	B	A	I	H	G	B	A	B	A	A	29	46,52–58
56	G	M	L	C	A	I	H	I	B	A	B	A	A	1	57
57	G	M	L	B	A	K	H	G	B	B	B	A	A	1	44
58	G	M	L	B	A	K	H	G	B	A	B	A	A	1	42
59	G	M	K	B	A	I	I	G	B	A	E	A	A	1	43
60	G	M	G	B	A	I	H	K	B	A	C	A	A	3	51
61	H	M	G	B	A	I	I	H	B	A	A	A	A	1	43
62	G	P	G	B	B	I	H	G	B	A	A	A	A	1	45
63	L	M	G	B	A	I	H	G	B	A	A	A	A	3	52,53
64	G	M	G	B	A	I	H	G	B	A	A	A	A	7	50
65	A	M	G	B	A	I	H	G	B	A	A	A	A	1	48
66	G	M	G	B	A	I	H	G	B	A	A	A	A	13	43,44,48,50,57,60
67	G	M	M	B	A	I	H	G	B	A	A	A	A	1	57
68	G	M	L	B	A	I	H	G	B	A	A	A	A	2	42,57
69	G	M	G	B	A	I	I	H	B	A	A	A	A	1	43
70	G	M	G	B	A	I	M	G	B	A	A	A	A	2	44,45
71	G	M	G	B	A	I	I	G	B	A	A	A	A	12	42–44,46,50,51
72	G	N	G	B	A	I	I	G	B	A	A	A	A	2	43,51
73	G	N	K	B	A	I	I	G	B	A	A	A	A	1	43
74	G	N	K	B	A	J	H	G	B	A	A	A	A	1	44
75	G	N	G	B	A	I	H	G	B	A	A	A	A	4	42,43,46,51
76	G	N	G	B	A	J	H	G	B	A	A	A	A	2	42
77	A	H	G	C	A	A	A	G	B	A	A	A	A	1	51
78	A	M	L	B	A	A	A	J	B	A	B	A	A	1	56
79	A	R	L	B	A	A	A	G	B	A	B	A	A	1	53
80	A	S	L	B	A	A	A	G	B	A	B	A	A	1	53
81	A	Q	L	B	A	A	B	G	B	A	B	A	A	1	50
82	A	Q	L	B	A	A	A	G	B	A	B	A	A	1	43
83	A	H	L	B	A	A	B	G	B	A	B	A	A	1	54
84	A	H	L	B	A	A	B	G	B	D	B	A	A	1	49
85	A	H	L	B	A	A	A	G	B	A	B	B	A	1	49
86	N	H	L	B	A	A	A	G	B	A	B	A	A	1	51
87	A	H	L	B	A	A	A	G	B	A	B	A	A	46	43–45,50,51,52–59
88	I	H	K	B	A	A	B	G	B	A	A	A	A	1	44
89	A	H	G	B	A	A	N	G	B	A	A	A	A	1	49
90	A	H	G	B	C	A	A	G	B	A	A	A	A	1	48
91	A	H	G	B	A	A	A	G	B	A	A	A	B	2	47,48
92	A	H	G	B	A	A	A	G	B	A	A	A	A	11	42,43,46,47,49,61, 62
93	J	H	G	B	A	A	A	G	B	A	A	A	A	1	44
94	A	M	G	B	A	A	A	G	B	A	A	A	A	4	42,60
95	A	O	G	B	A	A	B	G	B	A	A	A	A	4	43,63
96	A	H	G	B	A	A	B	H	B	A	A	A	A	1	44
97	A	H	G	B	A	A	B	G	B	A	A	A	A	9	42,43,45–47
98	A	H	G	B	A	A	G	G	B	A	A	A	A	4	42,43,46
99	A	T	G	B	A	A	A	G	B	A	A	A	A	1	61

compared with other North American ones, as documented previously from allozymes (Bodaly et al. 1991), was reflected in mtDNA variation.

The fact that mtDNA haplotypes of this Eurasian group that were found in Beringia are very closely related to others that were widely distributed in the Eurasian populations that we surveyed suggests that the recolonization of Beringia by this group occured in recent evolutionary times. Furthermore, very closely related mtDNA genotypes found in populations separated by over 10 000 km, contemporary

unconnected river drainages, and the marine Bering Strait imply an exceptional degree of dispersal and gene flow for freshwater fishes. Indeed, the levels of divergence documented among whitefish mtDNA genotypes found on two separate continents are characteristic of intrapopulation variation frequently observed in many animal groups, including fish (see literature in Avise 1989). Such a genetic structure contrasts sharply with the general observation that most freshwater fishes currently exhibit the highest levels of interpopulation gene diversity reported in any animal group (Gyllensten 1985). In fact, the broad scale continental and intercontinental geographic distribution of slightly differentiated phylogenetic assemblages observed in whitefish approaches the type of phylogeographic structure usually documented in species with life history conducive to dispersal and/or that occupy a range free of firm barriers to gene flow such as some marine organisms, bird species, and humans (Avise et al. 1992; Ball et al. 1988; Cann et al. 1987; Graves et al. 1984; Palumbi and Wilson 1990). This phenomenon most likely implies that whitefish have episodically been fragmented into several groups that subsequently dispersed extensively.

During the Pleistocene, huge glacial lakes persisted through most of Siberia, and the Beringian land bridge opened during each glacial maxima (Denton and Hughes 1981). River drainages flowing on an east–west axis developed on the bridge, which could have provided intercontinental dispersal routes for freshwater organisms (Lindsey and McPhail 1986). Whitefish of Eurasian origin may have been able to use these drainage connections to recolonize Beringia. However, in the absence of additional samples from Siberia, it is not possible at present to document the possibility that Beringian whitefish may have also used these routes to invade Eurasia, although their complete absence in Europe and our samples from Siberia (Lapin and Sos'va rivers) suggests that they never reached this region.

In contrast to the extensive dispersal of closely related haplotypes observed among northern European and Beringian populations, this study revealed that populations of northern and central Europe belonged to two phylogenetic groupings between which dispersal has been restricted. As both northern and central alpine Europe were episodically completely glaciated during the Pleistocene, both regions were most probably recolonized by two genetically distinct whitefish groups that evolved in allopatry in other regions, followed subsequently by limited intergradation. Vast areas of what is the present-day distribution range of whitefish in Eurasia remained unglaciated, especially in Siberia and were associated with extensive arctic and boreal climatic zones, expanding south to the Mediterranean coast (Flint 1971). Thus, these regions could have represented potential habitats for whitefish survival. Again, further sampling is needed in the eastern distribution range of whitefish in Eurasia to adequately document the historical biogeography of the species.

Globally, the broad-scale pattern of mtDNA variation among European populations resembled the pattern observed in North America outside Beringia where geographic populations occupied different branches of an intraspecific, phylogenetic tree. However, a major difference between North American and European (and Beringian) populations was the extremely reduced level of intrapopulation genetic diversity in North America. Bernatchez at al. (1989) argued that the dramatic difference in mtDNA diversity between anadromous whitefish populations from the Baltic Sea and Hudson Bay resulted primarily from population bottlenecks that correlated with the more important loss of potential habitat in North America than in Europe during Pleistocene glaciations. For instance, most of the North American continent north of the 40th parallel was ice covered during the Wisconsinian glacial advance. The arctic and boreal climatic zones, characteristic of the present-day distribution of whitefish, were telescoped within a relatively narrow band south of the ice sheet (Flint 1971; Fulton and Prest 1987), limiting refugia for cold-adapted species such as whitefish. This inevitably led to population reduction of cold-adapted fish species (McAllister et al. 1986), which may have resulted in an important loss of mtDNA genetic variability in North American whitefish populations. In contrast, vast areas of what is the present day distribution range of whitefish in Eurasia remained unglaciated, as described above. Thus, potential population reduction for whitefish in Eurasia may not have been as severe, resulting in a preservation of mtDNA variability.

Similarly, the difference in mtDNA diversity between Beringia, of which much remained unglaciated, and the rest of North America was attributed to differential population reduction through loss of habitat (Bernatchez and Dodson 1991). We propose that the generalized difference in mtDNA diversity between North American and Eurasian–Beringian populations is due to differences in the extent of population bottlenecks associated with differential glacier advance in different parts of the species range. Thus, low genetic diversity observed in North America corroborates the refugial concept, implying that population sizes in regions of endemism were drastically reduced for a period of time (Cracraft 1985). In contrast, the genetic divergence observed among distinct phylogenetic assemblages in Beringia and Europe suggested that several whitefish groups evolved in allopatry but does not provide evidence of any severe population reduction within each of these.

Lack of Congruence Between Genotypic and Phenotypic Variation in Whitefish

Our study indicates that traditional taxonomic units in whitefish do not reflect natural assemblages resulting from monophyletic divergence. Firstly, our results agreed with those obtained from allozymes in demonstrating that the major dichotomic distinction between *C. lavaretus* and *C. clupeaformis* based on continent of origin does not reflect the evolutionary history of whitefish. In fact, should such a distinction be maintained, Alaska–Yukon populations should naturally be included within the *C. lavaretus* complex (Fig. 3). However, because of the lack of diagnostic morphological distinction between both groups, and because levels of mtDNA variation (as in allozyme variation) observed between both continents are more representative of intraspecific variation (generally less than $P = 0.001$; see Billington and Hebert 1991 for a review), we suggest that *C. lavaretus* and *C. clupeaformis* should be recognized as a single, variable and circumpolar ranging species, *Coregonus lavaretus*, according to the most ancient name in use.

The taxonomic recognition among Eurasian populations is much more complicated due to the many examples of persistent reproductive isolation among morphologically distinct ecotypes, thus fulfilling a major criteria of the biological species concept (e.g., Svärdson 1979; Kirchhofer

and Tschumi 1986). Furthermore, the present study suggested that the morphological radiation observed within both northern and central Europe would have occurred following the last glacier retreat, some 18 000 yr ago. In addition, our results indicated numerous cases of polyphyletic and paraphyletic (distinct morphotypes genetically more closely related to each other than to identical ones from other regions) assemblages resulting from the traditional taxonomic designation. This casts doubts on the validity and utility of different taxonomic designations of whitefish populations based solely on morphological patterns.

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Comparison of Three Anadromous Rainbow Trout (*Oncorhynchus mykiss*) Populations Using DNA Fingerprinting and Mixed DNA Samples

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DNA fingerprinting of 15 individuals from three populations of steelhead trout (*Oncorhynchus mykiss*) was compared with DNA fingerprinting of mixtures of 15 individuals from the same populations. Three oligonucleotide fingerprinting probes were used to detect genetic variation in individuals and mixtures. Scanning image analysis coupled with customized software was used to assign band identity. The degree of band sharing of individuals within and between populations was then determined. Although band-sharing estimates were similar within and between populations, bands found predominately in one population were identified. DNA fingerprinting of mixtures detected bands found only in the majority of the individuals in the mixture, allowing differences between populations to be more readily identified.

Les empreintes génétiques (« DNA fingerprinting ») de 15 individus provenant de trois populations de truite arc-en-ciel (*Oncorhynchus mykiss*) ont été comparées à celles de mélanges de 15 individus provenant de la même population. Trois sondes oligonucléotidiques ont été utilisées pour détecter les variations génétiques chez les individus et dans les mélanges. L'analyse d'image couplée à un logiciel personnalisé a été utilisée pour déterminer l'identité des bandes. On a ensuite déterminé le degré de partage des bandes ches les individus à l'intérieur des populations et entre ces dernières. Bien que les résultats estimés relatifs au partage des bandes aient été semblables à l'intérieur des populations et entre les populations, les bandes appartenant de façon prédominante à une population ont été identifiées. Le « fingerprinting » des mélanges n'a décelé que les bandes que l'on retrouve dans la majorité des individus dans le mélange, ce qui a permis d'identifier plus facilement les différences entre les populations.

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Since the development of DNA fingerprinting (Jeffreys et al. 1985) researchers have recognized many potential applications of this technique. Use of DNA fingerprints for the identification of individuals in forensics is well established (Gill et al. 1985; Moody 1989). Researchers have also utilized DNA fingerprinting to determine paternity both in humans and in organisms for which mating systems were unclear (for review see Burke 1989). Although it is accepted that more closely related individuals should have more similar DNA fingerprints, only limited population-level studies have been carried out (Gilbert et al. 1990; Wirgin et al. 1991; Ellegren et al. 1992).

The application of DNA fingerprinting to fisheries science has been limited. Most DNA fingerprinting probes examined have produced DNA fingerprints in fishes (Fields et al. 1989; Lloyd et al. 1989; Rico et al. 1991; Turner et al. 1991). However, DNA fingerprinting of a sufficient subsample of individuals for population analysis is laborious and expensive. DNA fingerprints of salmonid fishes are complex and have numerous bands (Bentzen et al. 1991). In addition, even after the fingerprints have been generated, objective analysis is difficult.

Various approaches might be taken to simplify the analysis of DNA fingerprints. Single-locus fingerprint probes have

the advantages that only a few major bands are revealed and analysis of the resulting pattern is much easier (Taggart and Ferguson 1990; Bentzen et al. 1991). Single-locus probes are typically isolated by screening a library with a repeated element and then using each clone to probe a Southern blot. Unfortunately, only a small subset of the clones screened may reveal the desired degree of polymorphism. Thus, while single-locus probes produce a result that is easier to interpret, much effort must be put forth prior to obtaining a useful probe.

Another option to facilitate the analysis of complex fingerprint patterns is computer analysis. Scanning hardware and complementary software that can read X-ray films are currently available. These tools can be coupled with customized programs to generate a usable output. This can reduce the time required to analyze fingerprint data and provide objectivity not possible when scoring DNA profiles by eye.

Mixed DNA samples may also be useful in the comparison of populations. Dunnington et al. (1990) have demonstrated the utility of DNA fingerprint analysis of DNA mixtures in poultry breeding. Although these researchers studied inbred lines of fowl, the same principle of DNA mixing should hold true for outbred populations. Only those bands that occur in a majority of individuals in a given mix should be visible. All individual-specific bands should be diluted to the point that they are not detectable. The result is a DNA population profile that is comprised of bands found in most individuals in the population. Using one or some combination of these approaches should facilitate use of DNA fingerprint profiles for population-level analysis.

Materials and Methods

Sample Collection and Preparation

Blood was collected 1:1 in Alsever's solution (2.0% sucrose, 0.8% trisodium citrate, 0.4% sodium chloride) by cardiac puncture from anesthetized rainbow trout (*Oncorhynchus mykiss*). Rainbow trout were collected from the Dworshak National Fish Hatchery, located on the Clearwater River near Orofino, Idaho, and populations from two lower Columbia River tributaries, the Skamania State Trout Hatchery, located on the West Fork of the Washougal River in southwestern Washington, and the South Tacoma State Hatchery, located on Chambers Creek near Tacoma, Washington. Coho salmon (*Oncorhynchus kisutch*) were collected from the Big Qualicum Hatchery, located on the east coast of Vancouver Island, British Columbia. Blood samples were stored on ice for transport and either extracted upon arrival or stored at −20°C for future use. Genomic DNA was extracted from blood following the proteinase K – SDS digestion protocol described in Sambrook et al. (1989) omitting the phenol extractions. DNA was quantified by fluorometry and digested to completion with *Hae*III (New England Biolabs) following the manufacturer's directions. DNA mixes were prepared by mixing equal weights of 15 requantified digested DNA samples from each population. Two independent mixtures were prepared from the Dworshak population. All fish comprising the Dworshak mixtures were unique, with no fish occurring in both mixtures.

DNA Fingerprinting Techniques

For each lane, 2.5 μg of digested DNA was electrophoresed in 1× TAE at 50 V for 26 h in 0.7% agarose (Sambrook et al. 1989). Electrophoresis was conducted at 4°C to prevent overheating and the buffer was recirculated to avoid exhaustion of buffering capacity. DNA was transferred to a nylon membrane (Magnagraph, MSI) by capillary transfer in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) using ten sheets of Quick Draw blotting paper (Sigma). Oligonucleotides based on Jeffreys 33.6 core fragment (Jeffreys et al. 1985), a region of bacteriophage M13 (Vassart et al. 1987), and a sequence related to the *Drosophila* Per gene (Shin et al. 1985) were purchased amino modified and HPLC purified from Appligene. The sequences of the probes are as follows: 33.6, TGG AGG AGG GCT GGA GGA GGG C; M13, GAG GGT GGC GGT TCT; *Per*, $(ACA\ GGC)_3$. Probes were prepared by directly linking alkaline phosphatase to the oligonucleotide using the Lightsmith I TM kit from Promega. Membranes were prehybridized for 30 min at the temperature of hybridization (56°C for 33.6; 45°C for M13 and *Per*) in 10 mL of 2× SSC, 2% SDS, and 1% casein. Hybridization followed for 30 min in 5 mL of 5× SSC, 2% SDS, 10% polyethylene glycol, plus the appropriate probe at approximately 250 fmol/mL as recommended in the Lightsmith manual. Stringent washes were conducted at the temperature of hybridization; two sequential washes in 250 mL 1× SSC and 1% SDS were conducted followed by two sequential washes in 250 mL 1× SSC and 1% triton X-100. Membranes were then washed twice in 250 mL of room temperature 1× SSC. DNA fingerprints were visualized by equilibrating the membrane in 250 mL of carbonate buffer (50 mM sodium bicarbonate, 1 mM $MgCl_2$, plus sufficient 50 mM sodium carbonate, 1 mM $MgCl_2$ to reach a pH of 9.4) for 5 min, followed by the application of 1.5 mL of detection solution (5.3 μL AMPPD (Tropix)/mL carbonate buffer) and exposing the membrane to Fuji X-ray film. Exposures were typically for 7–14 h. Images were examined for population-specific bands by visually scanning the image for bands that appeared predominately in one population.

Membranes were then stripped and reprobed. Stripping was completed by washing the membranes twice with 250 mL of 1× SSC and 1% SDS at 94°C as described in the Tropix manual for chemiluminescent hybridizations. Membranes were then rinsed in room temperature 1× SSC and dried for storage. Prior to subsequent probings the membranes were hydrated in distilled water. Hybridizations were conducted as described above.

Computer Analysis of Fingerprint Patterns

Fingerprint patterns were analyzed by image analysis using a Microtek ScanMaker 600ZS and a Macintosh IIci computer. Images were converted to an array of molecular weights and intensities for each band using the NCSA GelReader program (Redman and Jacobs 1991). The accuracy of the scanner was tested by overlaying the image with an acetate sheet and marking each band with a permanent marker. Both the original image and the marker reproduction were scanned and the results compared. In our hands the scanner can identify approximately 90% of the existing bands. The remaining 10% can be manually added from within the GelReader program.

A presence–absence matrix was constructed from the array of molecular weights by utilizing a customized program interfaced with the GelReader program. Output format and allowed error tolerance are selected by the user. Error tolerances were empirically determined by alternating

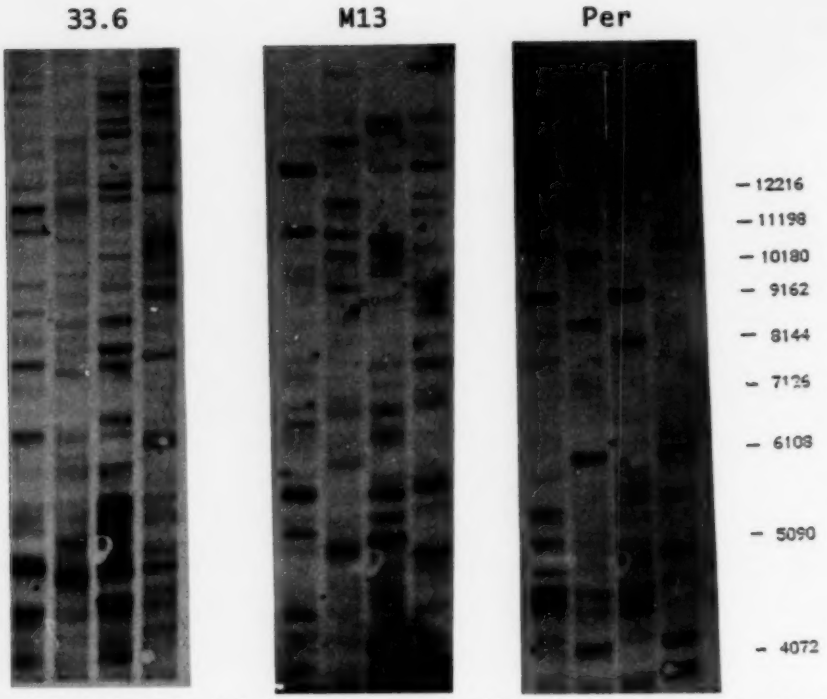

FIG. 1. Typical salmonid DNA fingerprints derived from three nonradioactively labeled oligonucleotide probes Jeffreys 33.6, M13, and Per. Molecular weights are given in base pairs.

TABLE 1. Average band-sharing indices derived by comparison of DNA fingerprint patterns of 15 individual rainbow trout from Chambers Creek, Dworshak, and Skamania hatcheries. Band-sharing comparisons between mixtures of 15 individuals from each of the three rainbow trout populations and one coho salmon population, Big Qualicum, are given in parentheses. Comparisons between Dworshak and the other populations are the average of two band-sharing indices from independent mixtures of 15 fish each from the Dworshak population.

Population	Probe	Chambers Creek	Dworshak	Skamania
Chambers Creek	33.6	0.438	0.426 (0.247)	0.449 (0.324)
	M13	0.309	0.319 (0.297)	0.312 (0.348)
	Per	0.322	0.337 (0.149)	0.420 (0.444)
Dworshak	33.6		0.556 (0.894)	0.456 (0.322)
	M13		0.387 (0.870)	0.347 (0.356)
	Per		0.447 (0.923)	0.318 (0.230)
Skamania	33.6			0.446
	M13			0.316
	Per			0.347
Big Qualicum	33.6	(0.235)	(0.197)	(0.162)
	M13	(0.296)	(0.113)	(0.154)
	Per	(0.000)	(0.149)	(0.000)

two individuals across one gel and obtaining eight molecular-weight estimates for each band. The error tolerance was defined as the difference between the maximal and minimal values divided by the average value for each band. The deviation increases with increasing molecular weight. To compensate for the weight-dependent deviation, the allowed error tolerance was adjusted to reflect the empirically determined value for each molecular weight. An error tolerance of 4% was determined for bands 20 kb in weight. This value decreased linearly to 2% for bands 1 kb in weight. Our values for error around computer-determined molecular weights are in accord with other published error estimates (Haig et al. 1993).

Bands were identified as being identical if their molecular weights were within the error tolerance identified for that molecular weight. In proceeding through the analysis the program identified the band with the highest molecular weight. If the second-largest fragment fell within the error-tolerance range it was identified as the same band. The molecular weights of these two bands were then averaged to

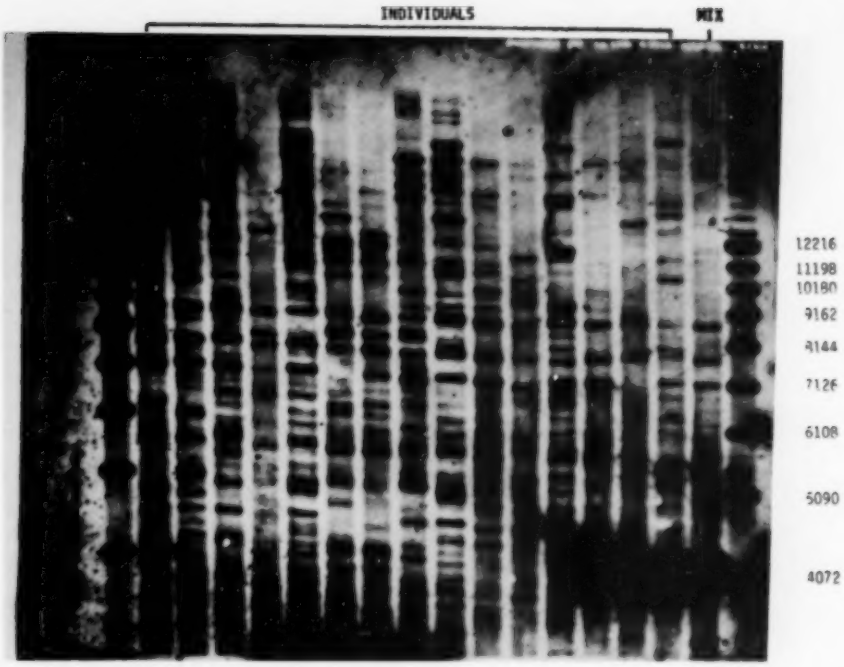

FIG. 2. Comparison of DNA fingerprints detected using M13 to probe 15 rainbow trout from the Dworshak population and the DNA fingerprint of a mixture of equal amounts of DNA from the same 15 individuals. Molecular weights are given in base pairs.

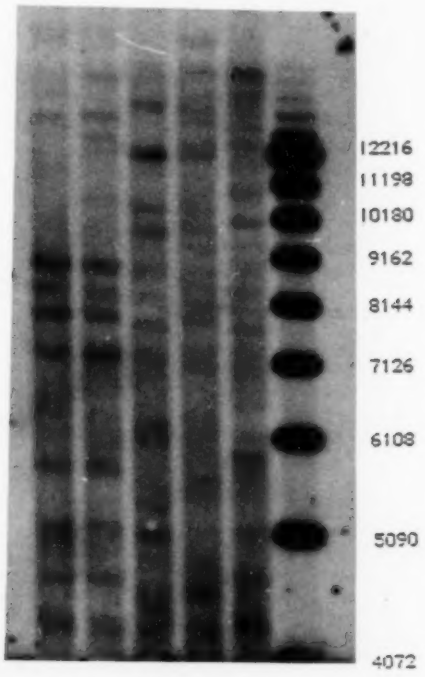

FIG. 3. DNA fingerprint of 15 mixed DNA samples from four populations using the M13 probe. From left to right, Dworshak steelhead mix 1, Dworshak steelhead mix 2, Skamania steelhead, Chambers Creek steelhead, and Big Qualicum coho. Molecular weights are given in base pairs.

obtain an adjusted molecular weight value. This new average was then compared with the third-largest fragment. Each time a new band was added to a group, a new average was calculated. When a band did not fall within the error tolerance for the molecular weight, it began a new molecular-weight size class. Bands assigned to the previous molecular-weight group were then reevaluated. If a band was more similar in weight to the average of the next molecular-weight group it was reassigned to that group. Each fragment from largest to smallest was evaluated in this manner. From these data a matrix was constructed identifying the presence or absence of a band for each molecular-weight class in each lane. A copy of this program will be made available on request.

From the presence–absence matrix a band-sharing index (BS) was calculated as $BS = 2N_{ab}/(N_a + N_b)$ (Wetton et al. 1987) where N_{ab} is the number of shared bands, N_a is the number of bands in one lane, and N_b is the number of bands in the other lane. Fifteen individuals from each population were used to calculate an average band-sharing index within and between populations. All pairs of individuals being compared were present on the same membrane. A minimum of 150 comparisons was used to estimate the average within- and between-population band-sharing indices. Band-sharing indices were also calculated for DNA mixtures by treating each mix as if it were an individual.

Results

The electrophoretic conditions described resolved all bands greater than 4 kb. All three probes produced readable DNA fingerprint patterns in this size range. A typical image

from each probe is shown in Fig. 1. Although no bands were found that were present in every individual of one population and none of another population, four bands were identified that were found in 11 or more of the 15 Dworshak fish examined but only 1 fish from each of the other two populations. In addition, 20 of 30 steelhead examined had a band of approximately 6.8 kb when probed with Jeffreys 33.6. This band was absent from the mixed coho sample.

Band-sharing indices calculated from individual and population comparisons are given in Table 1. The individual-based band-sharing indices are comparable within and between populations. The only observable trend is that the within-Dworshak value is consistently the highest for each probe. This may not be unexpected in that the Skamania and Chamber Creek populations originated from a mixture of stocks that inhabited southwestern Washington and Puget Sound regions, respectively (Thorgaard 1983). The Dworshak population originated from fish returning to the North Fork of the Clearwater River after the construction of Dworshak Dam. Thus, the fish used to establish the Dworshak population may have been more genetically similar than were the founders of the other two populations.

DNA mixtures produced from each population revealed bands found only in the majority of those individuals. A comparison of 15 individuals from the Dworshak population and the profile obtained from the mixture of these same individuals is shown in Fig. 2. Population profiles derived by probing all of the population mixes with M13 are shown in Fig. 3. Band-sharing indices found by comparing mixtures are also given in Table 1. Data from all three probes indicate that the two Dworshak population mixtures are virtually identical. The between-population band-sharing indices comparing the three steelhead populations to each other are similar. The band-sharing indices comparing the steelhead populations to the Big Qualicum coho population are generally lower than the between-steelhead indices.

Previous attempts to differentiate these steelhead populations have given conflicting results. Allendorf and Utter (1979) used allozyme data to conclude that Clearwater drainage and inland steelhead were less similar to coastal strains than the coastal strains were to one another. However, Dworshak and Skamania populations have commonalities in chromosome number not shared by the Chambers Creek population (Thorgaard 1983).

Discussion

DNA fingerprinting using mixed DNA samples successfully detected genetic variability between populations. Other researchers have used the band-sharing index proposed by Wetton et al. (1987) to estimate the genetic relatedness of individuals or have identified population-specific DNA fragments. These studies, however, have investigated either small isolated populations (Gilbert et al. 1990), potentially bottlenecked populations (Wirgin et al. 1991), or organisms that have been domesticated for an extended period (Ellegren et al. 1992). In contrast to those studies, we examined three large randomly bred populations. Although certain fingerprint bands were much more common in some populations than in others, within-population band-sharing indices were not significantly higher than between-population band-sharing indices. This probably results from coincidental matching of unrelated bands because of the high number of bands present. This individual variation is diluted using DNA mixtures, allowing differences between the populations to be more readily identified. Analysis of mixed DNA samples is also much quicker and easier than individual analysis and could be facilitated by mixing equal amounts of cell samples, such as blood or semen, prior to DNA extraction (Dunnington et al. 1992). In some cases, analysis of many individuals from multiple populations may not be possible. Comparison of DNA mixtures in these cases would allow an initial screen for genetic variability between populations. Although analysis of DNA mixes differentiated the populations we studied, we were not able to add resolution to the relationships among them. Given the rapid evolution of minisatellites, the populations surveyed may be at the limit of the resolving power of mixed DNA fingerprint analysis. This conclusion is supported by the similarity between the coho population and the three rainbow trout populations. Further studies including more closely related populations will clarify the utility and limitations of this approach.

Computer analysis of DNA fingerprints also facilitated the population comparisons. Image analysis allows objective scoring of bands and eliminates tedious scoring of DNA fingerprints by hand. Programs are currently available that assign a molecular weight to each band and customized programs can use these data to identify bands present in individuals or mixed samples. The initial cost of generating these programs may be quickly recovered in reduced labor time associated with analyzing the fingerprint profiles.

In addition to DNA mixes and computer analysis, the use of nonradioactively labeled fingerprinting probes should expand the use of this technique. Individual safety and convenience is increased, allowing researchers unfamiliar with radioactive techniques access to this technology. Numerous other fingerprinting probes could provide a researcher with abundant genetic markers without cloning or other technically demanding procedures (Mariat and Verguard 1992). The same probes we have used for this analysis yield less complex patterns in other fishes (Wirgin et al. 1991), further simplifying analysis. Nonradioactively labeled probes, DNA mixes, and computer analysis should expand the use of DNA fingerprinting and allow this technique to be more widely used for population analysis.

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DNA Fingerprinting and Cloning of Hypervariable Minisatellite Repeats in Salmonids

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We used heterologous Jeffreys' 33.6 core sequence and microsatellites $(CAC)_5$ and $(CA)_{12}$ as probes and compared them with probes based on the minisatellite sequences from tilapia (*Oreochromis niloticus*) and Atlantic salmon (*Salmo salar*) in fingerprinting assays. DNA fingerprints generated with the Jeffreys' 33.6 core sequence and the microsatellite $(CAC)_5$ and $(CA)_{12}$ probes showed complex profiles with high background, but DNA fingerprints using the tilapia and Atlantic salmon probes showed clear, less complex, informative, individual-specific DNA fingerprints suitable for analysis. We cloned and sequenced homologous repetitive sequences using a novel approach of creating a chinook salmon (*Oncorhynchus tshawytscha*) genomic DNA library with enriched low C_0t DNA repeats for the development of DNA probes. The four types of repeats identified and sequenced were $(CT)_n$ and three *Alu*-like sequences. We generated DNA fingerprints using one of the minisatellite sequences as a probe. This minisatellite sequence was shown to be species specific because it is abundant in chinook and coho salmon (*Oncorhynchus kisutch*) genomes, but not in Atlantic salmon. These probes will provide us with the tools to study pedigree and linkage analysis, paternity testing, breeding programs, and the analysis of genetic structure within populations for aquaculture and fisheries research.

Nous avons utilisé la séquence noyau hétérologue 33,6 de Jeffrey et les microsatellites $(CAC)_5$ et $(CA)_{12}$, servant de sondes et les ont comparées, dans des analyses de cartographie d'ADN, à des sondes en séquences minisatellites obtenues du tilapia (*Oreochromis niloticus*) et du saumon de l'Atlantique (*Salmo salar*). Les empreintes digitales d'ADN obtenues à l'aide de la séquence noyau 33,6 de Jeffrey et des sondes microsatellites $(CAC)_5$ et $(CA)_{12}$ montrent des profils complexes, mais les empreintes d'ADN obtenues à l'aide des sondes du tilapia et du saumon de l'Atlantique étaient claires, moins complexes, informatives et caractéristiques de l'individu, se prêtant ainsi à l'analyse. Les auteurs ont cloné des séquences homologues répétitives et en ont établi les séquences selon une nouvelle approche, soit en créant une bibliothèque d'ADN génomique du saumon quinnat (Oncorhynchus tshawytscha) à l'aide de répétitions enrichies d'ADN pour la mise au point de sondes en ADN faible en C_0t. Ils ont identifié et établi la séquences de quatre types de répétitions, soit $(CT)_n$ et trois séquences de type Alu. Ils ont en outre obtenu des empreintes d'ADN en se servant d'une des séquences minisatellites comme sonde, et ont démontré que cette séquence était caractéristique de l'espèce parce qu'elle était abondante dans les génomes des saumons quinnat et coho, (*Oncorhynchus kisutch*), mais non du saumon de l'Atlantique. Ces sondes pourront servir d'outils aux analyses de généalogie et de parenté, aux recherches de paternité, à l'évaluation des programmes de sélection de géniteurs et à l'analyse de la structure génétique de populations à des fins d'élevage et de recherche halieutique.

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Isozyme protein electrophoresis markers have traditionally been used by fisheries scientists to study genetic variation between hatchery and wild fish populations (Aebersold et al. 1987; Davis et al. 1990). Recently, restriction fragment length polymorphism and sequence polymorphism of mitochondrial DNA (mtDNA) have provided additional genetic markers for measuring genetic relatedness. However, mtDNA analyses do not always provide individual genetic identification, and the ability to detect population diversity using only mtDNA can be limited (Waldman et al. 1988; Smith et al. 1989; Shields et al. 1992). The advent of DNA fingerprinting has allowed scientists in fisheries and aquaculture research to develop genetic markers that are more polymorphic than those revealed by conventional isozyme electrophoresis and mtDNA analyses (Wright 1993).

The lack of suitable specific probes for fish genetic analysis has slowed the introduction of DNA fingerprinting into fisheries applications. Using heterologous probes from human *Alu*, M13, and others, DNA fingerprints have been generated for a variety of fish (Wright 1993). Jeffreys' probes 33.6 and 33.15 have been used to fingerprint tilapia (*Oreochromis niloticus*) (Harris et al. 1991; Bentzen et al. 1991), rainbow trout (*Oncorhynchus mykiss*), and Atlantic salmon (*Salmo salar*) (Bentzen et al. 1991). Fields et al. (1989) used M13 as a probe and successfully fingerprinted rainbow trout, chum salmon (*Oncorhynchus keta*), coho salmon (*Oncorhynchus kisutch*), and Atlantic salmon. Using the M13 probe, Rico et al. (1991) fingerprinted the threespine stickleback (*Gasterosteus aculeatus*) and Wirgin et al. (1991) fingerprinted

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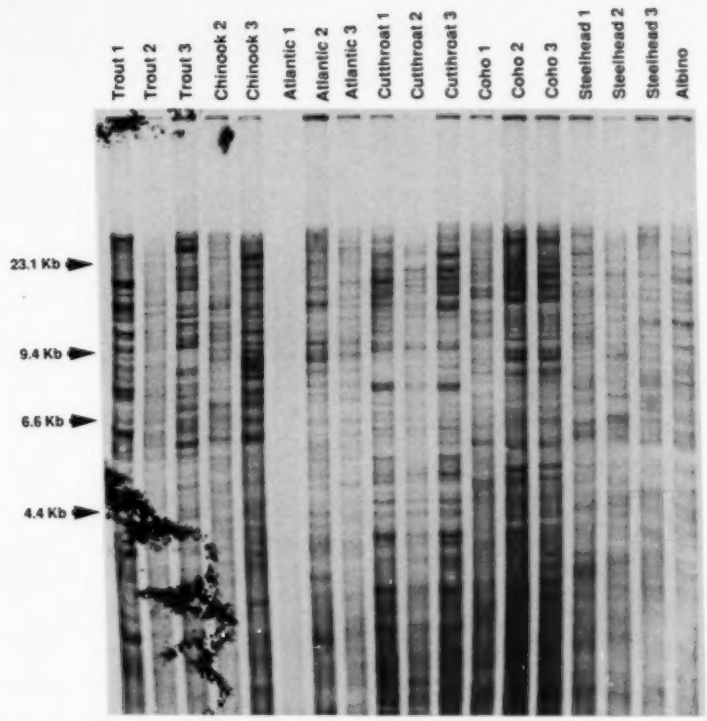

FIG. 1. DNA fingerprints of five species of salmonids. DNA from five species of salmonids was digested with Alu I and probed with 33.6 core. Trout = rainbow trout, Chinook = chinook salmon, Atlantic = Atlantic salmon, Cutthroat = Yellowstone cutthroat trout, Coho = Coho salmon, Steelhead = steelhead.

the striped bass (*Morone saxatilis*). Lloyd et al. (1989) used BKm minisatellites as a probe and revealed DNA polymorphism in the rainbow trout. Additional microsatellite probes such as $(CAC)_5$, $(GACA)_4$, $(CA)_8$, and others (Epplen et al. 1991) have been used in other fish species, including *Poecilia* sp. and *Xiphophorus* sp. However, the cross hybridization of these heterologous probes has usually generated fingerprints that contain numerous crowded bands of varying intensity, making interpretation and analysis difficult.

Repetitive sequences including satellites, minisatellites, microsatellites, short interspersed elements (SINE), and long interspersed elements (LINE) are found in all eukaryotic genomes (Brutlag 1980; Singer 1982; Miklos 1985). They have been extensively studied in mammals (Singer 1982; Arnason and Widegren 1984; Miklos 1985, Chen et al. 1989) and recently in fish (Datta et al. 1988; Moyer et al. 1988; Wright 1989; Denovan and Wright 1990; Bentzen and Wright 1993). These repetitive sequences have attracted considerable interest as genetic markers for chromosome mapping and phylogenetic studies (Rohrer et al. 1994; Fanning et al. 1989). Using a homologous minisatellite variable number of tandem repeats (VNTR), Bentzen and Wright (1993) generated clear and highly polymorphic DNA fingerprints in salmonid fish.

In this paper, we reported the DNA fingerprinting of salmonids using five probes: Jeffreys' 33.6 core sequence, microsatellite $(CAC)_5$ and $(CA)_{12}$, a tilapia minisatellite and an Atlantic salmon minisatellite. In addition, we report on the cloning of hypervariable minisatellite repeats in chinook salmon and on the use of these repeats as a probe in DNA fingerprinting.

Materials and Methods

DNA Isolation

Blood samples were obtained from the University of Washington and from the National Marine Fisheries Service (both in Seattle, Washington). DNA was extracted from five species of salmonids including coho salmon, chinook salmon, Atlantic salmon, Yellowstone cutthroat trout, steelhead and rainbow trout. Rainbow trout samples, including albino trout, were also collected from the Pacific Northwest Laboratory hatchery (Richland, Washington).

The blood samples were stored in EDTA anticoagulant vacutainers at 4°C for 48 h and then in 350-μL aliquots at −80°C. DNA was isolated according to a modified Federal Bureau of Investigation protocol (Kirby 1992). Blood samples were thawed at 37°C and resuspended in 800 μL of 1 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) with gentle vortexing. Cells were pelleted at 8000 × *g*, the supernatant removed and the pellet resuspended in 1 mL 1 × SSC, as before. Cells were pelleted again and resuspended in 375 μL of 0.3 M sodium acetate (pH 5.2). Sodium dodecyl sulfate (SDS), 0.5%, and 300 μg proteinase K (Sigma) were added to the suspension and mixed by inversion. Cells were incubated at 55°C with periodic agitation until the pellet was fully dissolved, usually for 1.5–2.0 h.

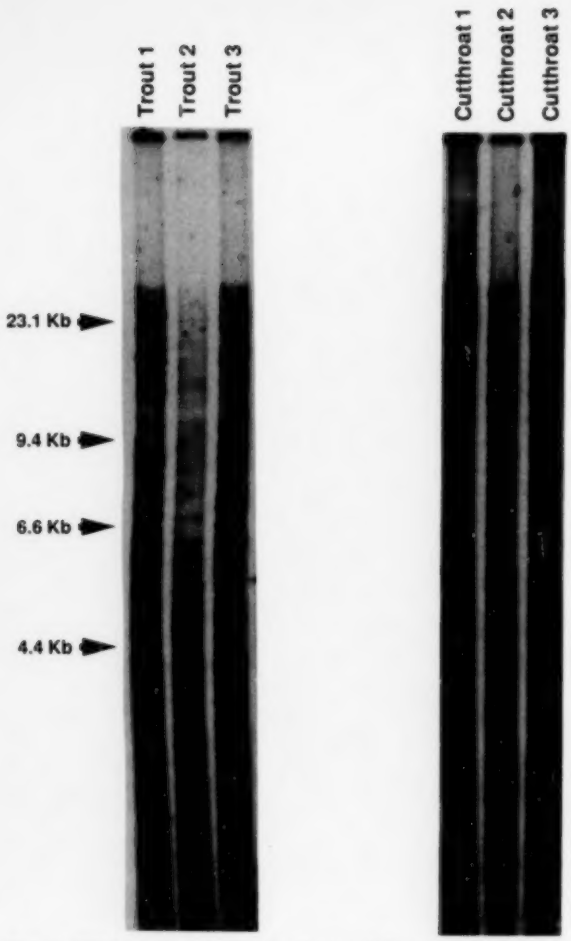

FIG. 2. DNA fingerprints from three rainbow trout and three Yellowstone cutthroat trout. DNA was digested with *Alu*I and probed with $(CAC)_5$.

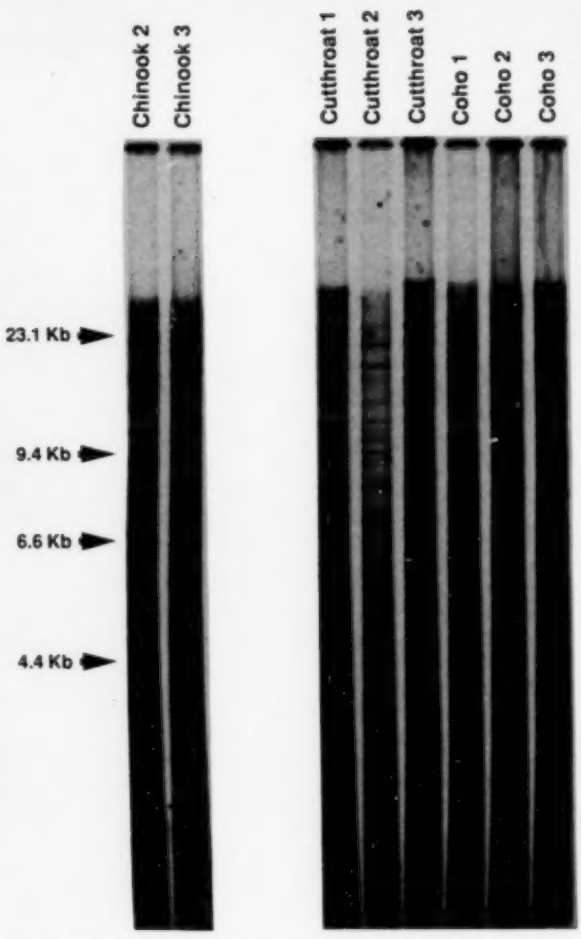

FIG. 3. DNA fingerprints from three Yellowstone cutthroat trout, two chinook, and three coho salmon. DNA was digested with *Alu*I and probed with $(CA)_{12}$.

When digestion was complete, 480 μL of 10 mM Tris, 1nM EDTA (TE) (pH 7.8) buffered phenol was added and mixed by inversion for at least 30 s, and the phases were separated with a 2-min spin at 16 000 × *g* in a microcentrifuge. The bottom organic layer was removed with a glass pipette, and the remaining aqueous layer extracted two more times with an equal volume of water-saturated phenol – chloroform – isoamyl alcohol (25:24:1), followed by two extractions with an equal volume of chloroform – isoamyl alcohol (24:1). The remaining aqueous layer was transferred to a new tube and nucleic acids precipitated by adding 1 mL of ice-cold absolute ethanol and incubating at room temperature for 10 min. DNA was recovered by centrifugation at 16 000 × *g*, and the pellet was redissolved in 180 μL of TE. Nucleic acids were then reprecipitated with 1/10 volume of sodium acetate (3 M, pH 5.2) and ethanol and the resulting pellet was washed with 70% ethanol and dried under vacuum for 5 min. The final DNA pellet was redissolved in TE and quantified by Hoescht 33582 staining with a Hoefer TKO minifluorometer.

Enzyme Digestion and Electrophoresis

Ten micrograms of genomic DNA were digested overnight with 50 units *Alu*I, *Hae*III or *Hinf*I (GIBCO/BRL, Gaithersburg, MD) in 100-μL total volume. Digested DNA was precipitated with ammonium acetate, washed with 70% ethanol, dried under vacuum, and resuspended in 20-μL of TE. DNA was separated in 0.7% agarose gels (Seakem GTG, FMC) in 40 mM Tris-acetate, 1mM EDTA (TAE) (pH 8.0) buffer at 1.5 V/cm, 6°C for 48 h and was transferred to Magnagraph nylon membranes (MSI Inc.) using a Pharmacia VacuGene XL blotting system and the acid depurination protocol supplied by the manufacturer. Nucleic acids were fixed to the nylon membrane by UV crosslinking in a Stratagene model 1800 UV Stratalinker at 1200 mJ/cm^2. Membranes can be stored indefinitely when dry and at room temperature.

Probe Preparation

Oligonucleotide probes were synthesized on an Applied Biosystems 392 DNA Synthesizer with an amino-modified

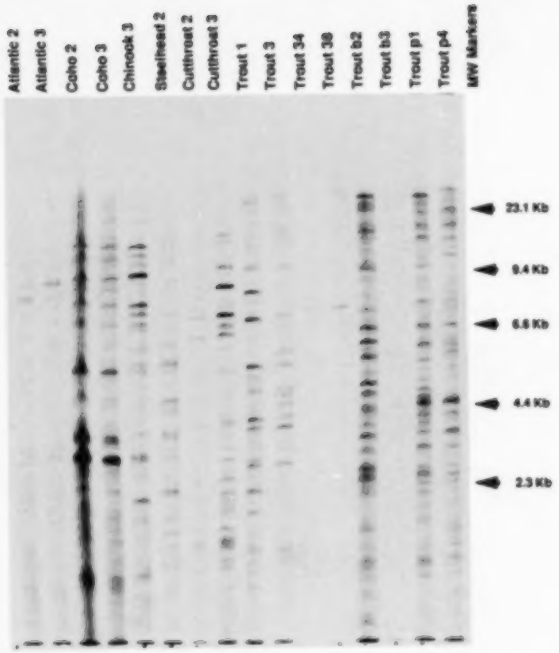

FIG. 4. DNA fingerprints of five species of salmonids. DNA from five species of salmonids was digested with *Hinf*I and probed with a tilapia minisatellite sequence probe. Trout = rainbow trout; Chinook = chinook salmon; Atlantic = Atlantic salmon; Cutthroat = Yellowstone cutthroat trout; Coho = coho salmon; Steelhead = steelhead.

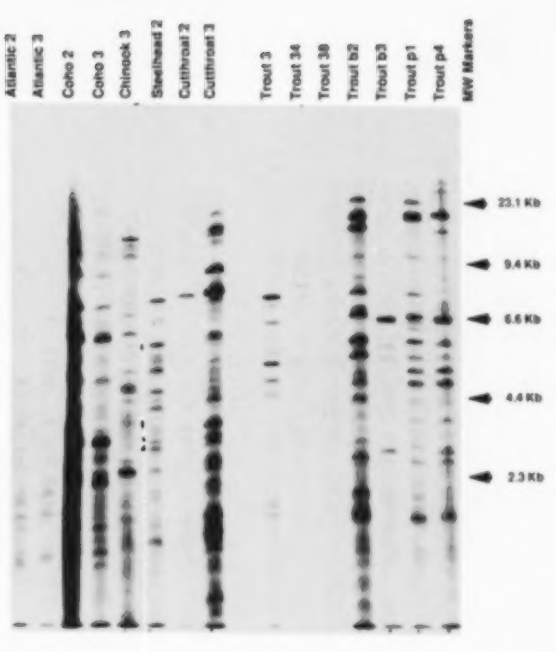

FIG. 5. DNA fingerprints of five species of salmonids. DNA from five species of salmonids was digested with *Hae*III and probed with a tilapia minisatellite sequence probe. Trout = rainbow trout; Chinook = chinook salmon; Atlantic = Atlantic salmon; Cutthroat = Yellowstone cutthroat trout; Coho = coho salmon; Steelhead = steelhead.

base at the 5′ end (Aminolink, ABI). Alkaline phosphatase (AP) was conjugated directly to the primary amine with a Promega Lightsmith kit according to the manufacturer's instructions. Oligonucleotide probes included Jeffreys' 33.6 core sequence, $(CAC)_5$, $(CA)_{12}$, a tilapia minisatellite sequence (Wright 1989; Franck and Wright 1993), an Atlantic salmon minisatellite sequence (Bentzen and Wright 1993), and a chinook salmon minisatellite sequence obtained from Table 2b.

Hybridization and Chemiluminescent Detection

Dried membranes were placed inside glass hybridization tubes (Robbins Scientific Model 310 Hybridization Incubator) and prehybridized for 20 min at the appropriate temperature in 5 × SSC, 0.5% BSA, 1% SDS (33.6 core at 55°C, $(CAC)_5$ at 35°C, $(CA)_{12}$ at 55°C, tilapia probe at 55°C, Atlantic probe at 42°C, and chinook probe at 60°C). Prehybridization solution was decanted and replaced with 5 mL of the same solution containing DNA probe at concentration ranged from 0.5 to 1.0 pmol/mL. Hybridization occured for 20 min and the hybridization temperature was probe specific at the temperatures listed above. Two washes were performed for 5 min using 1 × SSC, 1% SDS, and two more washes were performed for 5 min with 1 × SSC, 1% Triton X-100 at the temperature of hybridization. Membranes were then transferred to a clean plastic container and were washed twice for 5 min each with 0.3% Tween 20 in 1 × PBS (0.4 M Na_2HPO_4, 0.1 M NaH_2PO_4, 1.0 M NaCl, pH 7.4) at room temperature. Membranes were then washed once in assay buffer (0.1 M diethanolamine, 1 mM $MgCl_2$) for 5 min and then covered for 10 min with 15 mL of assay buffer containing 60 μg/mL of Lumigen PPD chemiluminescent substrate (Lumigen Inc., Detroit, MI). Damp membranes were wrapped in plastic wrap and immediately exposed to Kodak XAR-5 film for 1–3 h. After detection and scoring of bands, the membranes were stripped of the probe by washing four times for 5 min each with boiling 0.1 × SSC, 0.1% SDS, followed by a room temperature rinse with 2 × SSC. Stripped membranes were stored at 4°C for reprobing.

Construction of Chinook Low C_0t Genomic Library

Genomic chinook DNA (~440 μg) was sheared with a sonicator into fragments ranging in size from 200 bp to 2.5 kb. The sheared DNA was then heat denatured by boiling and was incubated at 60°C for 7 min. Double-strand DNA (dsDNA) was separated from the single-strand DNA with a hydroxylapatite column. The recovered dsDNA was then blunt-ended and cloned into pUC 18 and transformed into *E. coli*. The pUC 18 library was screened with the 33.6 core sequence as a probe. One of the clones that hybridized to the 33.6 core sequence probe was subjected to sequence analysis in both orientations by the dideoxy chain termination method (Sanger et al. 1980).

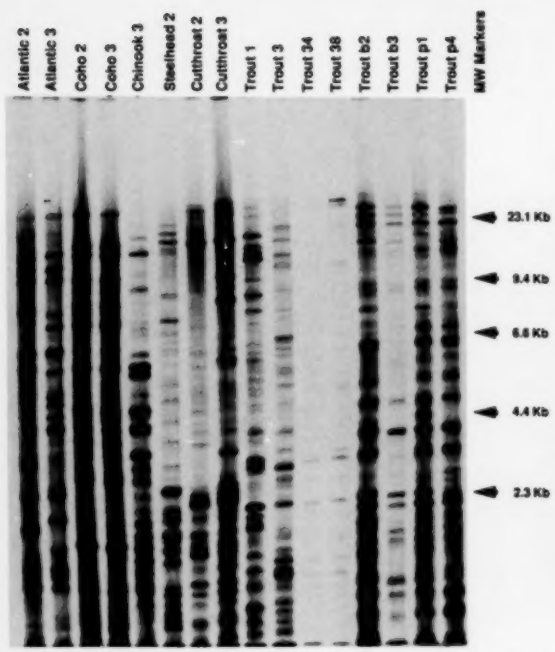

FIG. 6. DNA fingerprints of five species of salmonids. DNA from five species of salmonids was digested with *Hinf*I and probed with an Atlantic salmon minisatellite sequence probe. Trout = rainbow trout; Chinook = chinook salmon; Atlantic = Atlantic salmon; Cutthroat = Yellowstone cutthroat trout; Coho = coho salmon; Steelhead = steelhead.

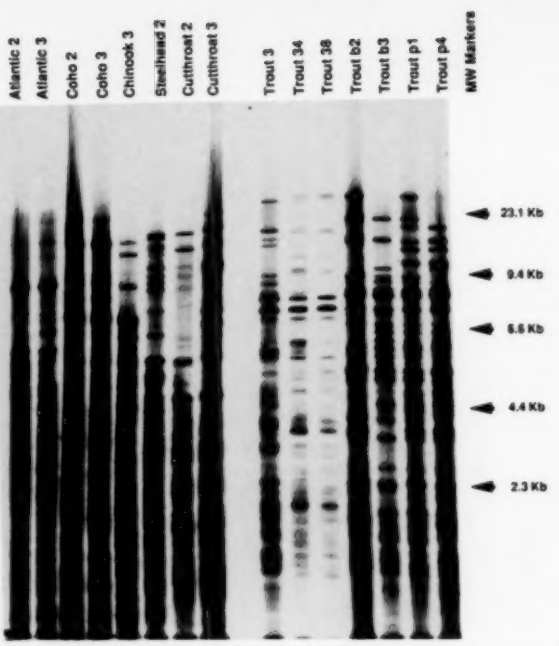

FIG. 7. DNA fingerprints of five species of salmonids. DNA from five species of salmonids was digested with *Hae*III and probed with an Atlantic salmon minisatellite sequence probe. Trout = rainbow trout; Chinook = chinook salmon; Atlantic = Atlantic salmon; Cutthroat = Yellowstone cutthroat trout; Coho = coho salmon; Steelhead = steelhead.

Results

Using Jeffreys' 33.6 core sequence as a probe, complex (ranging from 20 to 40 bands per lane) and highly variable fingerprints were obtained from five salmonid species. This high variability along with high levels of background are common when using a heterologous probe. However, we optimized the hybridization temperature and stringency during washing to significantly improve the overall fingerprint for generating individual specific fingerprints in all five species (Fig. 1). There were even higher backgrounds in the fingerprints when the microsatellites $(CAC)_5$ and $(CA)_{12}$ were used as probes in the assay (Figs. 2 and 3). When the tilapia and the Atlantic salmon minisatellite sequences were used as probes, clear polymorphic DNA fingerprints were obtained from all five salmonid species.

Autoradiograms with salmonid genomic DNA digested with *Hinf*I and *Hae*III and probed with the tilapia minisatellite probe are shown in Figs. 4 and 5. Autoradiograms with salmonid genomic DNA digested with *Hinf*I and *Hae*III and probed with the Atlantic salmon minisatellite probe are shown in Figs. 6 and 7. In general, DNA fingerprints generated with either the tilapia or the Atlantic salmon minisatellite sequence probes showed clear and less complex profiles compared with the fingerprints generated with the Jeffreys' 33.6 core sequence or the microsatellite $(CAC)_5$ and $(CA)_{12}$ sequence probes.

We used this fingerprinting assay to identify the parents of unexpected albino rainbow trout in our hatchery (Fig. 8). DNA from three of the albino offspring and the four potential parents was fingerprinted and analyzed. Because our broodstock is a more closely related group than an outbred population, we expect more than 50% band sharing between parents and offspring. We determined that fish AP-1 and AP-4 were the parents of the three albino offspring, AB-1, AB-2, and AB-3.

From the pUC 18 library containing the repetitive sequences from the Chinook genomic DNA, we sequenced one of the clones that hybridized to the 33.6 core sequences. The sequences of the insert are shown in Table 1. Four types of repeats were identified within this insert and are shown in Figs. 2a–2d. One type of repeat is characterized as a dinucleotide repeat of $(CT)_n$. The other three repeats containing the C/GAGG motif that hybridized to the 33.6 core sequence probe are as follows: TAA TAT AAT GAC AGT AGA TGG TAG ACC AGG G; GGA GAG AGG GAG CAG G; and GAG G.

We generated a chinook minisatellite AP probe based on the sequence listed on Table 2b and used it to fingerprint the five salmonid species genomic DNA cut with *Hinf*I and *Hae*III (Figs. 9 and 10). Clear DNA fingerprints were obtained with the trout, steelhead, and cutthroat DNAs. Minimum hybridization was observed with Atlantic salmon DNA. High intense hybridization (smear) without clear DNA profiles were seen with the chinook and coho salmon DNA. These data suggest that many copies of this minisatellite sequence are widely distributed within the chinook and coho salmon genomes but not in Atlantic salmon, suggesting that

TABLE 1. Nucleotide sequence of a colony from a Chinook salmon low C_0t DNA pUC library.

```
          10          20          30          40          50          60
ACT CTA GAG GAT CCC CGG GTA ATA TAA TGA CAG TAG ATG GTA GAC CAG GGT AAT ATA GTG

          70          80          90          100         110         120
ACA GTA GAT GGT AGA CCA GGG TAA TAT AAT GAC AGT AGA GGG TAG ACT GGT ACT CTC TCT

          130         140         150         160         170         180
CAT CTC TCT CTC TCG TCT CTC TTG TCT CTC TCT CTG TCT CTC TCT GTC TCT CGG CTT GTC

          190         200         210         220         230         240
ACC AAA AGC ACT CAC AAA CTT CTA CAG ATG CAC AAT CGA GAG CAT CCT GGC GGG CTG TAT

          250         260         270         280         290         300
CAC CGC CTG GTA CGG CAA CCG TTC CGC CCA CGA GGG AGG GAG GAG AGA GGG AGC AGG GGA

          310
GAG AGG GAG GAG GGC A
```

the chinook sequence from Table 2b is a species-specific minisatellite sequence.

Discussion

We demonstrated the use of heterologous probes for DNA fingerprinting individuals in salmonid fish. A common problem in using the 33.6 core sequences and microsatellite sequences of $(CAC)_5$ and $(CA)_{12}$ as probes is the high background in the fingerprints as reported by other investigators (Wright 1993). We were able to reduce general background by altering the stringency of hybridization temperature and posthybridization washes so that a clear and informative multilocus fingerprint was obtained. However, the application of minisatellite sequences from tilapia, Atlantic salmon, and chinook salmon as DNA probes gives clear, precise, low background, and less complex fingerprints that can be easily used for analysis.

Because most of the probes used in this study detect between 2 and 40 bands per DNA lane, it is possible to identify species specific markers. However, the use of these probes as definitive species and population markers must be validated with a larger sample size. Most importantly, since each probe used in DNA fingerprinting hybridizes to different regions of the genome, the application of population markers must be carefully analyzed and characterized. The ability of the DNA fingerprinting assay to identify individuals in a closely inbred population and to identify of paternal and maternal relationships proves the power of this approach in genetic identification. In fact, when a well characterized probe is used, it is feasible to use DNA fingerprinting as a genetic marker of hatchery fish, therefore providing a genetic data base for fisheries management and practices.

We demonstrated that the use of fish repetitive sequences as probes is the most practical approach in developing informative DNA fingerprints. Bentzen et al. (1994) used a homologous probe based on the tandem repeats in an Atlantic salmon minisatellite and obtained clear and highly informative multilocus DNA fingerprints for salmonid fish. Stevens et al. (1993) reported the cloning of a multilocus B2-2 probe from a chinook salmon genomic DNA library, and they compared fingerprints using the B2-2 multilocus and single-locus Atlantic salmon 3.15.34 probes. Using the B2-2 probe, 8 to 20 fragments were detected in any given DNA lane among five families of chinook salmon. They demonstrated that the B2-2 probe detected autosomal, unlinked, predominantly heterozygous DNA fragments that were inherited in a Mendelian fashion.

Some of the reported repetitive sequences show species and genus specificity and have been used for phylogenetic studies. Naruse et al. (1992) isolated and characterized a highly repetitive interspersed sequence, OLR1, from genomic DNA of medaka, *Oryzias latipes*. They found that the OLR1 sequence is conserved in three other species, *Oryzias lozonensis*, *Oryzias curvinotus*, and *Oryzias mekongnensis* but not in *Oryzias minutillus*, *Oryzias celebensis*, *Oryzias melastigma*, *Oryzias javanicus*, *P. formosa*, and *P. sphenops*. Goodier and Davidson (1993) isolated a repetitive sequence from the Atlantic salmon and showed the sequence to be highly species specific. Only brown trout (*Salmo trutta*) DNA hybridized to the Atlantic salmon repetitive sequence and no hybridization occurred with other related species including Arctic charr (*Salvelinus alpinus*), Japanese white-spotted char, brook trout (*Salvelinus fontinalis*), rainbow trout, chum salmon, chinook salmon, pink salmon (*Oncorhynchus gorbuscha*), coho salmon, masu salmon (*Oncorhynchus masou*), and Arctic grayling (*Thymallus arcticus*).

Murata et al. (1993) isolated several short interspersed elements and used the polymerase chain reaction to determine the phylogenetic relationships among Pacific salmonids. Their deduced data suggest three evolutionary lines: a line of chum salmon, pink salmon, and kokanee (*Oncorhynchus nerka*); a line of coho salmon and chinook salmon; and a line of steelhead trout. Their data also support the change in the phylogenetic assignment of steelhead trout from *Salmo* to *Oncorhynchus*. Kido et al. (1991) used the tRNA-derived retroposons repetitive sequences to substantiate the phylogenetic relationships among salmonid species. When we

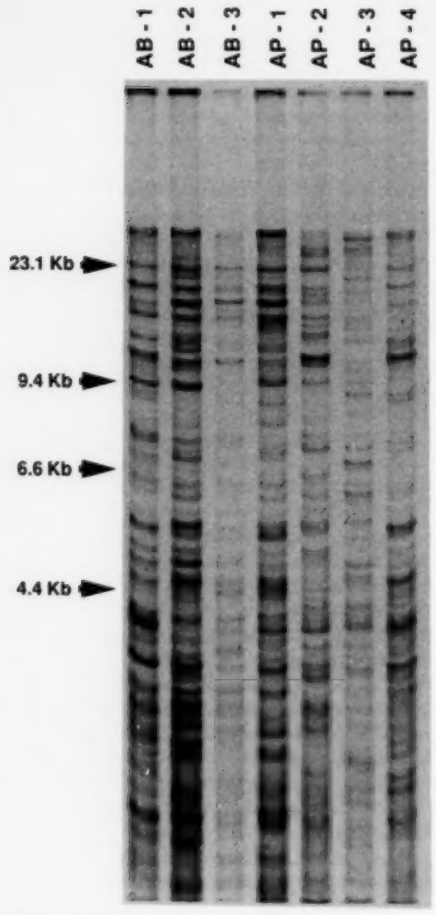

FIG. 8 DNA fingerprints from three albino rainbow trout and four suspected albino parents. DNA was digested with *Alu*I and probed with 33.6. AB = albino brood; AP = suspected albino parents.

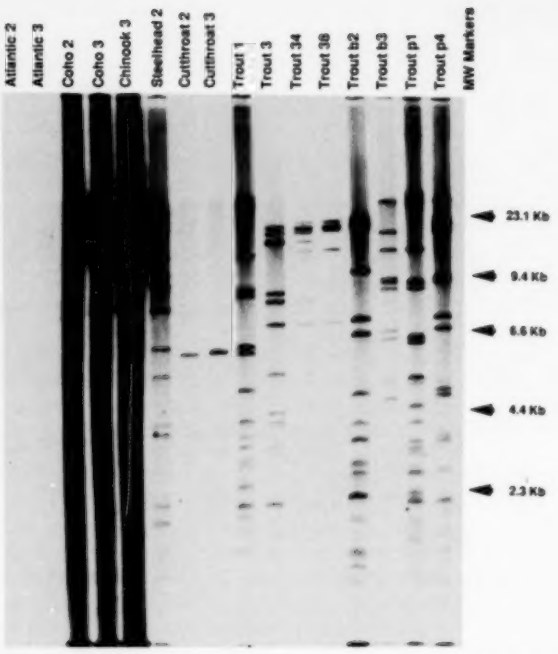

FIG. 9. DNA fingerprints of five species of salmonids. DNA from five species of salmonids were digested with *Hinf*I and probed with a chinook salmon minisatellite sequence probe. Trout = rainbow trout; Chinook = chinook salmon; Atlantic = Atlantic salmon; Cutthroat = Yellowstone cutthroat trout; Coho = coho salmon; Steelhead = steelhead.

TABLE 2. Nucleotide sequences of four different repeats from Chinook salmon. The underlined G in 2b represented a single bp substitution.

(a)
$(CT)_4$ CAT $(CT)_5$ CGT $(CT)_3$ TGT $(CT)_5$ GT $(CT)_4$
(b)
TAA TAT AAT GAC AGT AGA TGG TAG ACC AGG G
TAA TAT AGT GAC AGT AGA TGG TAG ACC AGG G
TAA TAT AAT GAC AGT AGA GGG TAG AC
(c)
GGA GAG AGG GAG CAG G
GGA GAG AGG GAG GAG G
(d)
GAG G
GAG G
GAG G

used our chinook repetitive sequence (Table 2b) as a probe in a DNA fingerprinting assay, we demonstrated that the chinook and coho genomes contain many copies of this repeat and the trout and Atlantic salmon contain fewer copies, suggesting a close genetic relationship between coho and chinook salmon among the five species of salmon Figs. 9 and 10). These data suggest that the use of repetitive sequences is one of the best available methods for clarifying the phylogenetic order of closely related species.

Most researchers obtain the minisatellite VNTR loci by first constructing a genomic library and subsequently screening it with the 33.6 and 33.15 probes (Bentzen et al. 1991; Taggart and Ferguson 1990). Repetitive sequences are found in all eukaryotic genomes (Brutlag 1980; Singer 1982; Miklos 1985), and they are characterized by their annealing kinetics (Britten and Kohne 1968). We circumvented these time consuming and labor intensive approaches by creating a pUC library with the enriched repetitive sequences isolated from genomic DNA. Repetitive sequences were enriched from total genomic DNA by their annealing temperature characteristics which we term low-C_0t DNA.

We successfully used the low-C_0t DNA as a probe to distinguish different species of pathogenic fungi (Leung et al. 1992). Our strategy allows faster access to the sequence of

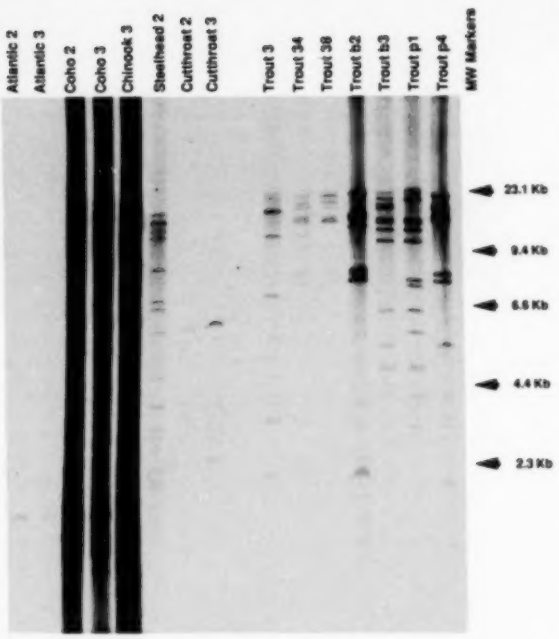

FIG. 10. DNA fingerprints of five species of salmonids. DNA from five species of salmonids was digested with *Hae*III and probed with a chinook salmon minisatellite sequence probe. Trout = rainbow trout; Chinook = chinook salmon; Atlantic = Atlantic salmon; Cutthroat = Yellowstone cutthroat trout; Coho = coho salmon; Steelhead = steelhead.

many, but not all, of the VNTR loci. We identified four types of repeats from one insert. Three of the repeats contained the CGAGG or GGAGG motif and one contained a dinucleotide $(CT)_n$ repeat. We then generated a synthetic oligonucleotide probe based on one repeat (Table 2b) and used it as a probe in DNA fingerprinting. This repetitive sequence is widely distributed within the chinook and coho genomes, resulting in smears in the fingerprints (Figs. 9 and 10), but not in rainbow trout, which give clear and precise DNA fingerprints. This approach enabled us to quickly develop DNA probes for detecting highly variable regions of the genome in fish species. These probes will then provide us the tools to study pedigree and linkage analysis, paternity testing, breeding programs, and the analysis of genetic structure within populations.

Identification and characterization of minisatellite VNTRs in salmonids will definitely offer better probes for DNA fingerprinting. Even though the biological functions of these VNTRs remain to be determined, they will offer immediate help in quantitative genetic studies in fisheries and aquacultural sciences. Recently, Bentzen and Wright (1993) reported an evolutionarily conserved minisatellite VNTR in Atlantic salmon demonstrating the use of VNTRs for studying fish molecular evolution. Application of DNA fingerprinting technology in fisheries is still in its infancy. Sequence information represents the data base for genetic analysis, but rapid development of new technology will make it simple to apply DNA fingerprinting to the study of aquaculture genetics and fisheries biology problems.

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Identification of Farm-reared and Native Chinook Salmon (*Oncorhynchus tshawytscha*) on the West Coast of Vancouver Island, British Columbia, Using the Nuclear DNA probe B2-2

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Withler, R.E., T.D. Beacham, R.F. Watkins, and T.A. Stevens. 1994. Identification of farm-reared and native chinook salmon (*Oncorhynchus tshawytscha*) on the west coast of Vancouver Island, British Columbia, using the nuclear DNA probe B2-2. Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 267–276.

The chinook salmon DNA probe B2-2 was used to distinguish farm-reared (from two commercial farms) and native chinook salmon (*Oncorhynchus tshawytscha*) that were sampled from five populations on the west coast of Vancouver Island. The Big Qualicum River population (east coast of Vancouver Island), which is believed to be the main progenitor of domesticated broodstocks used for aquaculture in British Columbia, was also sampled. The presence or absence and integrated optical densities (IODs) of three DNA fragments at 8.3 kilo base pairs (kbp) (band 1), 6.5 kbp (band 2), and 5.6 kbp (band 3) in the hybridization patterns of B2-2 on *Bam*HI-restricted DNA were recorded for 269 chinook salmon. The frequency of occurrence of bands 2 and 3, and all seven measurements made of the relative and absolute values of the IODs of the three bands, varied significantly among populations. The IOD of band 3 provided the best discrimination among populations. The Big Qualicum and two fish farm populations were differentiated from all five west coast native populations. Discriminant analysis and a neural network were used independently to classify correctly to type an average of 97% of the native and 83% of the farm west coast DNA patterns used as test samples.

La sonde d'ADN du saumon chinook B2-2 a été utilisée pour distinguer les saumons chinook d'élevage (provenant de deux établissements piscicoles commerciaux) et les saumons chinook indigènes (*Oncorhynchus tshawytscha*) qui ont été prélevés dans cinq populations sur la côte ouest de l'île de Vancouver. La population de la rivière Big Qualicum (côtes est de l'île de Vancouver) que l'on croit être la principale source de géniteurs dans les stocks domestiqués utilisés dans l'industrie de l'aquaculture en Colombie-Britannique a également été échantillonnée. On a enregistré, chez 269 saumons chinook, la présence ou l'absence ainsi que les densités optiques intégrées (DOI) de trois fragments d'ADN de 8,3 kilo paires de bases (kpb) (bande 1), 6,5 kpb (bande 2) et 5,6 kpb (bande 3), dans l'autoradiogramme d'hybridation de la sonde B2-2 sur de l'ADN découpé par *Bam*HI. La fréquence d'apparition des bandes 2 et 3 et la totalité des sept mesures des valeurs relatives et absolues des DOI des trois bandes ont varié de manière statistiquement significative d'une population à l'autre. La DOI de la bande 3 a fourni le meilleur indice pour distinguer les populations. Les populations de Big Qualicum et des deux établissements piscicoles ont été distinguées de la totalité des cinq population sauvages de la côte ouest. L'analyse de discrimination et un réseau neuromimétique ont été utilisés indépendamment pour classer correctement, jusqu'au type, en moyenne 97% des fragments d'ADN des poissons sauvages et 83% des fragments d'ADN des poissons d'élevage et de la côte ouest utilisés comme échantillons à analyser.

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Net-pen aquaculture of salmonid fishes has increased dramatically in British Columbia in the past decade. In 1990, the farmed production of primarily Atlantic salmon (*Salmo salar*) and chinook salmon (*Oncorhynchus tshawytscha*) increased 30% over 1989 levels to 15 000 t (Commercial Fisheries Section 1992). Since that time, the annual production of farmed fish has reached 20 000 t (British Columbia Salmon Farmers' Association 1992), compared with a 10-year average annual catch of wild salmon in British Columbia of 80 000 t (Department of Fisheries and Oceans 1991). The aquaculture industry is centered on Vancouver Island, with farms concentrated in the northern waters of the east coast and in protected inlets along the west coast. Escapes of farmed fish occur in Vancouver Island waters when net pens are damaged by storms, tidal action, navigational errors, predators, and vandalism. Fish also escape during transfer and handling. Once fish have escaped, recovery is seldom feasible, and escaped salmonids are known to enter spawning streams (Gausen and Moen 1991) and spawn among themselves or with native fish (Lura and Saegrov 1991; Webb et al. 1991). While farming of the exotic Atlantic salmon in British Columbia raises a number

of ecological concerns (Wing et al. 1992), intensive aquaculture of the native chinook salmon is not without genetic risk to wild populations (Hindar et al. 1991; Taylor 1991).

Initially, the chinook salmon cultured in British Columbia were obtained as fertilized eggs from salmon enhancement hatcheries operated by the Department of Fisheries and Oceans (DFO). The development of domesticated strains from farm-reared fish originally obtained from DFO was undertaken in the early 1980s by several private aquaculture operations. Broodstock were selected from several brood years of the Big Qualicum River stock and, to a lesser extent, the Robertson Creek stock. Farm-reared chinook salmon adults from other native B.C. populations were also available to industry on a more sporadic basis (Withler et al. 1987). In 1983, the milt from sex-reversed female (genetically female, phenotypically male) Big Qualicum fish became available to industry (Donaldson 1986). This "monosex" milt contains only X chromosome bearing sperm and produces all-female progeny (Hunter et al. 1983). Use of the Big Qualicum monosex milt has been widespread throughout the industry, with most chinook salmon farm operators switching to all-female production. During the 1980s, as many as 30 million chinook salmon smolts were stocked to net pens each year (D. Groves, Sea Spring Salmon Farm, personal communication). The annual level of stocking has declined greatly since that time, to 3.2 million in 1992 (British Columbia Salmon Farmers' Association 1992), owing both to the improved survival of farmed fish, and to the increased use of Atlantic salmon in B.C. aquaculture.

Although the chinook salmon cultured in B.C. are recently derived from native populations, their genetic composition has been altered to an unknown extent by founder effects, interpopulation hybridization, genetic drift, intentional and inadvertent selection during domestication, and, possibly, the effects of hatchery supplementation on the wild stocks used as founders. The indication that domesticated broodstocks are predominantly of Big Qualicum heritage is of concern because aquaculture operations are concentrated in waters adjacent to the natal streams of chinook salmon stocks of substantial commercial and sport fishery value (Argue et al. 1983). Chinook salmon populations on northeastern and western Vancouver Island are genetically distinct from the Big Qualicum stock (Utter et al. 1989). Given the considerable body of evidence that the extensive biochemical genetic differentiation observed among salmonid populations is accompanied by considerable adaptation to local environments (Ricker 1972; Taylor 1991), it is not surprising that intraspecific hybridization generally leads to a loss of productivity in wild populations (Hindar et al. 1991). Thus, the potential entry of large numbers of escaped farm-reared fish of Big Qualicum origin into the river systems of northeastern and western Vancouver Island is of major concern regardless of the extent of genetic alteration that has occurred in their short domestication history.

The application of maximum likelihood (ML) methods to genetic data provided by protein electrophoresis can provide accurate estimates of the proportional contributions of chinook salmon populations to a mixed-population sample (Brodziak et al. 1992). However, ML methods of stock identification do not classify individual fish to population of origin. In this study, we have combined the use of a nuclear DNA probe that demonstrates substantial stock-specificity in chinook salmon with two methods of mixed-population analysis that allow classification of individuals: discriminant analysis and neural network analysis. We used the B2-2 DNA probe isolated from chinook salmon (Stevens et al. 1993) to characterize five native populations of chinook salmon, and two domesticated chinook salmon populations from commercial fish farms that have experienced fish escapes on the west coast of Vancouver Island, and the Big Qualicum River chinook salmon population on the east coast of Vancouver Island. The objective was to find fixed genetic differences between the native and cultured fish that could be used to detect escaped farm fish in west coast Vancouver Island streams, and identify the purebred and hybrid progeny of escaped fish that spawn successfully in local streams.

Materials and Methods

DNA Samples

Between 1987 and 1992, samples of liver tissue were collected from between 25 and 50 mature chinook salmon from enhancement facilities at each of Nitinat River, Conuma River, Kennedy River, Robertson Creek, and Thornton Creek on the west coast and at Big Qualicum River on the east coast of Vancouver Island (Fig. 1). In 1992, liver tissue samples were collected from 25 mature farm chinook salmon from fish farm 1, and from 50 immature farm salmon (weight range 1–3 kg) from fish farm 2 in the Clayoquot Sound region of the west coast of Vancouver Island (Fig. 1). In all cases, the liver tissue samples were frozen and genomic DNA was extracted subsequently (Stevens et al. 1993).

Southern Blot Analysis

Genomic DNA from each fish was restricted with the enzyme *Bam*HI. The concentration of the restricted DNA was measured spectrophotometrically, and 3.0 μg samples were electrophoresed on 30-well, 0.6% agarose gels for 720 $V \cdot h^{-1}$. *Hin*dIII-restricted λ DNA size markers were run in four lanes of each gel, and two standard fish were run on all (Robertson Creek 18 (RC18)) or most (Big Qualicum 12 (BQ12)) gels. Methods of transfer of DNA to nylon membranes and hybridization with the [α^{32}P]dATP-labelled B2-2 probe were as described by Stevens et al. (1993).

Image analysis of the autoradiographs was carried out using Bioimage software (Millipore Corp. Imaging Systems, Ann Arbor, Mich.) on a SPARC workstation. Autoradiographs were photographed with a Kodak CCD camera at a resolution of 1024 × 1024 pixels following equalization and calibration of the camera to ensure accurate band size and optical density measurements. The Bioimage software was used to create a molecular weight standard network on each image from the four lanes of λ *Hin*dIII fragments (23.1, 9.4, 6.6, 4.4, 2.3, and 2.0 kilo base pairs (kbp)) to estimate the molecular weight of all bands between 2.3 and 9.4 kbp detected on the image. The area and average optical density (OD) of every band in each lane on the image was calculated and used to estimate the optical density integrated over band area (IOD) for each band. The sum of the IODs of all bands in each lane provided the lane IOD for each DNA sample. Estimated molecular weights of the 15 DNA fragments of the two standard fish, RC18 and BQ12, from all images were used to measure the variation among images in the determination of molecular weight for various size classes of DNA fragments (see below). Similarly, RC18 band and lane

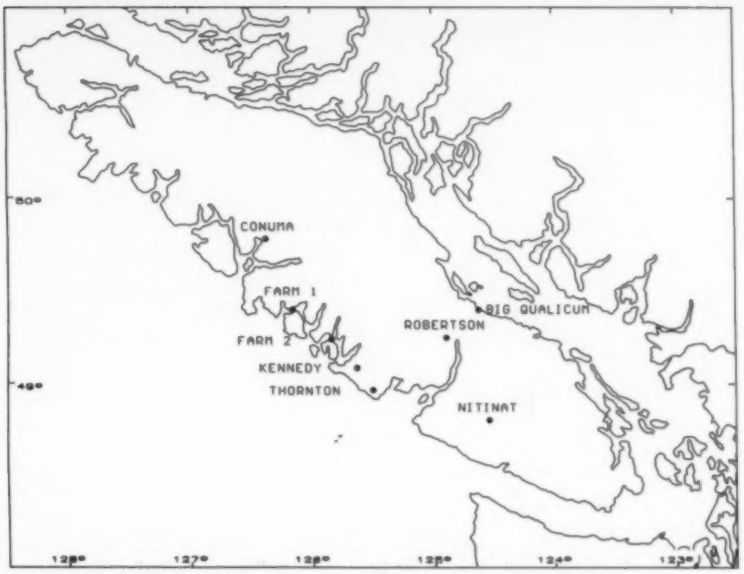

FIG. 1. Locations of chinook salmon populations sampled in this study. Native populations were sampled from the west coast (Robertson Creek, Thornton Creek, Nitinat River, Conuma River, and Kennedy River) and east coast (Big Qualicum River) of Vancouver Island. The two fish farms were located in the Clayoquot Sound area.

IOD measurements from all images were used to correct band IOD values for variation in DNA sample concentration within images, and for variation in the specific activity of the probe and autoradiograph exposure times among images (see below).

On *Bam*HI-restricted chinook salmon DNA, B2-2 hybridizes strongly to DNA in two molecular weight ranges. Between 2.4 and 2.7 kbp, B2-2 hybridizes to a band doublet of 2.60 and 2.65 kbp in every chinook salmon, and to a 2.45-kbp fragment in over 95% of all chinook salmon examined. Depending on the exposure level of the autoradiograph, the doublet is detected on an image as either one or two bands. Comparisons of the doublet band intensity with band intensities produced by single locus probes in chinook salmon indicate that B2-2 binds to multiple copies of the 2.60- to 2.65-kbp fragments in all fish and that the number of binding sites is uniform among chinook salmon. In contrast, B2-2 hybridization to DNA fragments between 5.4 and 8.7 kbp is polymorphic among chinook salmon. Within this size range, each fish possesses up to three fragments to which B2-2 hybridizes strongly, at 8.3 kbp (band 1), 6.5 kbp (band 2), and 5.6 kbp (band 3). B2-2 also hybridizes less strongly with additional *Bam*HI-restricted chinook salmon DNA fragments intermediate in size to the monomorphic fragments between 2.4 and 2.7 kbp and the polymorphic fragments between 5.4 and 8.7 kbp.

Within-image Sample Standardization

Although the spectrophotometric readings indicated that 3 μg of DNA was loaded in each lane of all gels, variation in band intensity among fish on the same image suggested that a standard loading of 3 μg of high molecular weight DNA was not achieved. Repeated runs of individual samples revealed that the variation was due primarily to incorrect determination of DNA concentrations among samples rather than to variability in the volume of restricted DNA loaded on a gel. Thus, because the RC18 samples loaded on every gel were drawn from a single DNA sample, relatively uniform quantities of RC18 DNA (putatively 3 μg) were loaded on each gel. Because B2-2 hybridizes with equal intensity to the DNA of the 2.60 and 2.65-kbp doublet in every chinook salmon, the band IOD of the doublet (quantified as a single band) in each fish compared with the band IOD of the doublet of the RC18 standard fish was used to standardize the IODs of all bands on the image to a uniform DNA loading (that of RC18).

For the standardization, samples containing 0.5, 1.5, 3.0, 4.5, and 6.0 μg of DNA from each of six fish were loaded on a single gel. Five classes of band intensity were defined on the basis of the proportion of lane IOD represented: <5% lane IOD, 5–10, 10–20, 20–30, and >30%. For the six fish, the bands between 5.4 and 8.7 kbp (i.e., bands 1, 2, and 3) and the 2.60 and 2.65-kbp doublet were quantified over the range of sample concentrations. For bands of each intensity class, the band IOD values were regressed on the natural logarithm of the IOD of the 2.60- to 2.65-kbp doublet of the same fish. This provided, for each intensity class, the relationship between band and doublet IODs over a range of sample concentrations. Because a relatively constant amount of DNA from RC18 was loaded on every gel, we standardized the IOD of each band of each sample on an image to the RC18 doublet IOD of that image by classifying the band to the appropriate initial intensity class and using the following equation:

$$(1)\quad IOD_s = IOD_o + b_i(\log IOD_{RC18,\ 2.6\text{-kb band}} - \log IOD_{observed\ 2.6\text{-kb band}})$$

where IOD_s is the integrated optical density of a band standardized to the IOD of the RC18 doublet, IOD_o is the observed IOD of a band, b_i is the slope of the regression for the intensity class of the band being standardized (i = 1–5), $IOD_{RC18,\ 2.6\ kb}$ is the observed IOD of the RC18 doublet, and $IOD_{observed\ 2.6\text{-kb band}}$ is the observed IOD of the doublet

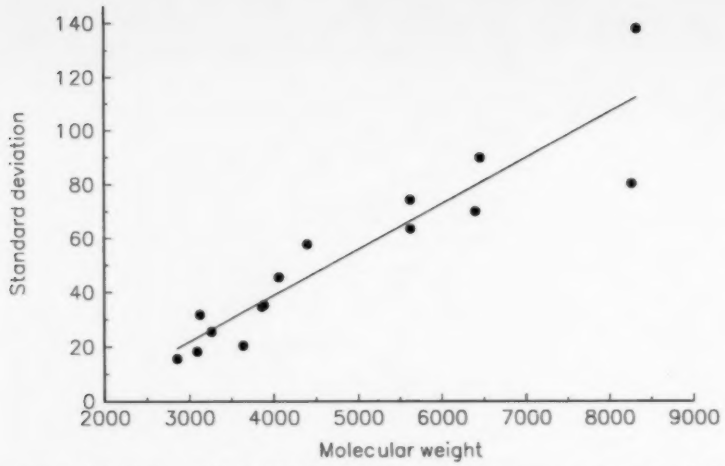

FIG. 2. Regression of the standard deviation of band size (molecular weight) measurements on band size for 15 DNA fragments between 2.8 and 8.3 kbp detected by the B2-2 probe in two standard fish run on all (Robertson Creek 18) or most (Big Qualicum 12) gels.

of the fish whose bands are being standardized. This standardization was based on the assumption that the intensity of the 2.60 and 2.65-kbp doublet would be the same for all fish if 3 μg of high molecular weight DNA actually had been added to all lanes.

Among-image Sample Standardization

Band intensities varied among images owing to a number of factors, such as the specific activity of the probe, the autoradiograph exposure time, and the number of intensifying screens used. We standardized band IODs among images in a manner similar to the standardization among fish within images. We developed three autoradiographs with differing band intensities from a single blot containing DNA samples from 26 chinook salmon from the study, including RC18. The autorads were typical of "light," "medium," and "dark" autorads produced in the study, as judged by the lane IOD values for RC18. For each of the five intensity classes of band IOD previously defined, we regressed the band 1, 2, and 3 IODs of fish on the natural logarithm of the lane IOD of RC18 for the three images. This provided, for each intensity class, the relationship between band IOD and RC18 lane IOD over a range of autorad intensity levels. Thus, individual band intensities, after within-image standardization to the RC18 doublet IOD, were then standardized to the mean of RC18 lane IOD over all images. This was done using the equation

$$(2)\quad IOD_t = IOD_s + b_i(\log(\text{mean lane } IOD_{RC18}) - \log(\text{observed lane } IOD_{RC18}))$$

where IOD_t is the band IOD standardized to a common RC18 lane IOD, IOD_s and b_i are as previously defined, mean lane IOD_{RC18} is the mean lane IOD of RC18 over all images (67.81), and observed lane IOD_{RC18} is the lane IOD for RC18 on the particular image being standardized. Bands with a transformed IOD value of less than 0.05 were judged to be too light to be detected consistently, and thus were assigned a value of 0.00 in the analysis.

Molecular Weight Bins for Band Classification

The precision of our estimation of band size (molecular weight) was determined by calculating the mean and standard deviation of the repeated size measurements of seven RC18 and eight BQ12 fragments among images. We regressed the standard deviation on fragment size ($F = 76.29$, $P < 0.01$, $n = 15$) (Fig. 2) to determine the measurement variation for fragment sizes between 2.5 and 9.0 kbp. A binning procedure (Gill et al. 1990; Galbraith et al. 1991) was used to determine if a particular band was present. Bin widths were set at about 7 (for bands 1 and 3) or 14 (for band 2) standard deviations on the basis of the RC18 and BQ12 observations. The bin boundaries for band 1 were 7.95 and 8.75 kbp, for band 2 were 6.2 and 7.2 kbp, and for band 3 were 5.4 and 5.8 kbp. The wider bin width for band 2 was chosen because of an apparently real greater variability in fragment size for bands of this size class. The standardized optical densities of all bands present in a bin (0, 1, or 2) were summed to determine the IOD value.

Data Analysis

Seven variables were determined for each fish in the analysis: the standardized IODs of bands 1, 2, and 3; the band 2/band 1, band 3/band 1, and band 3/band 2 IOD ratios; and the sum of the band 1, 2, and 3 IODs divided by the total lane IOD. We calculated generalized squared distances (Pimentel 1979) among the Big Qualicum population, the two fish farm populations, and the five west coast of Vancouver Island populations using the DISCRIM procedure in the SAS statistical package (SAS Institute Inc. 1989). A dendrogram from the matrix of the distances was constructed using the UPGMA option in the CLUSTER procedure in SAS. Stepwise discriminant analysis was conducted using SAS to identify the variables that enabled discrimination among populations, and between farm and native west coast chinook salmon.

Two methods of identification of farm fish among the west coast of Vancouver Island samples were employed: discriminant analysis and development of a neural network. All fish analyzed from the five west coast of Vancouver Island populations as well as the two farm sites were pooled and 20% of the sample (46 individuals) was randomly picked without replacement and assigned for testing; the remaining 80% of the sample (186 individuals) was used to provide the baseline for discrimination of farm from native chinook

salmon. This procedure was repeated five times to create five independent sets of training and test fish for use in both the discriminant analysis and the neural network. The mean and standard deviation of the percent correct classification of farm-reared and native fish was determined for both methods. Discriminant analysis was conducted using SAS, and the neural network analysis was conducted using Brainmaker Professional from California Scientific Software (Nevada City, Calif.). Each neural net was constructed using three layers (input layer, hidden layer, and output layer). The input layer had 7 neurons, the hidden layer 10 neurons, and the output layer 2 neurons (farm or native). Training tolerance was set at 0.1 and the networks were allowed to run to completion or, if that was not possible, until 90% of the training sample was accurately classified. The test fish were then classified as farm-reared or native depending on which value from the network was higher.

Results

Banding Patterns and Sample Standardization

The B2-2 probe, when hybridized to chinook salmon DNA restricted with *Hae*III, provides a multilocus fingerprinting pattern that reveals considerable genetic variability among individual fish within populations (Stevens et al. 1993). However, on *Bam*HI-restricted DNA, the pattern of B2-2 hybridization was very different. The 2.60 and 2.65-kbp doublet occurred in all chinook salmon, but for fragments between 2.7 and 9.4 kbp the pattern of B2-2 hybridization was polymorphic (Fig. 3). Between 5.4 and 8.7 kbp, variation occurred both in band presence and absence and, for those bands that were present, in band intensity (as measured by band IOD). High resolution electrophoresis of DNA samples has revealed that multiple fragments occur in some fish within each of the band 1, 2, and 3 molecular weight bins that may reflect variable number tandem repeat (VNTR) variability (R.E. Withler, and T.A. Stevens, unpublished data). Under the electrophoretic and autoradiographic exposure conditions of this study, the multiple bands within each molecular weight bin generally could not be differentiated, and only a single band, or no band, was quantified for a fish within each molecular weight bin. Occasionally, two distinct bands were detected within the bin width used to define bands 1, 2, and 3, and the IODs of both bands were summed and used in the analysis. Differences among fish, and among populations, in the band 1, 2, and 3 IODs may have resulted from variability in the number of distinct fragments present within each bin, the copy number of each of those fragments, and the degree of homology between the B2-2 probe and the binding sites of each of the fragments.

The standardization of samples within images, using Equation 1, was carried out for 20 individual images among which the observed mean of the 2.60- to 2.65-kbp doublet IOD ranged from 23.90 to 45.30. The mean coefficient of variation of doublet IOD values over all images was 24.56, and ranged from 11.92 to 45.48 for individual images. The standardization of samples among images was carried out using Equation 2. The mean RC18 lane IOD over all images was 67.81, and ranged from 38.0 to 129.2.

Banding Pattern Differences Among Populations

After standardization of band intensities (IODs) for within- and among-blot variation in sample quantification and autoradiographic conditions, all chinook salmon examined in this study were found to possess one or more of the three DNA fragments that hybridize to B2-2 at 8.3 kbp (band 1), 6.5 kbp (band 2), and 5.6 kbp (band 3) (Table 1). Band 1, present in 98% of the fish, was detected most often, whereas band 3 was the fragment least frequently detected in the *Bam*HI-restricted DNA samples. Significantly different proportions of fish among populations possessed bands 2 (χ^2 = 31.10, df = 7, $P < 0.05$) and 3 (χ^2 = 167.47, df = 7, $P < 0.001$), but not band 1 (χ^2 = 8.93, df = 7, $P > 0.05$). The mean band IODs for bands 1, 2, and 3, for those fish that possessed each band, were variable among populations (Table 1). Similarly, the pairwise ratios of band IODs for fish that possessed both of each pair of bands varied among populations (Table 1). Thus, populations differed both in the proportions of fish that possessed bands 2 and 3, and in mean band 1, 2, and 3 IODs for those fish that did possess each band.

The differentiation among populations resulting from both differences in band frequencies and band IODs is apparent in the values presented in Table 2. In this case, the mean band IODs and pairwise band IOD ratios for each population were calculated over all fish in the population. For band IODs, fish lacking a band were assigned a value of 0. For IOD ratios, a value of 0 was assigned when the numerator band, or both bands, was absent. An arbitrarily high ratio value of 5.0 (see Table 1) was assigned when the denominator band only was absent. Significant variation occurred among populations in the IOD values of band 1 ($F_{[7,261]}$ = 16.94, $P < 0.001$), band 2 ($F_{[7,261]}$ = 5.56, $P < 0.001$), and band 3 ($F_{[7,261]}$ = 71.19, $P < 0.001$) (Table 2).

For band 3, there was a correlation between band frequency and band IOD. For those populations with a low frequency of this band, the band 3 IOD in those fish possessing the band was also low (Table 1). As a result, the mean band 3 IOD values for the Conuma, Nitinat, and Kennedy populations calculated over all fish in each population were very low (Table 2). The intensities of all three bands tended to be higher in the Big Qualicum and fish farm populations than in the west coast Conuma, Nitinat, and Kennedy populations. Band 1 and 3 intensities of the Robertson and Thornton populations tended to be intermediate to, and their band 2 intensities greater than, those of the two previous groups (Table 2). These differences in mean band IODs are reflected in the values indicating the proportion of lane IOD attributable to the sum of IODs for bands 1, 2, and 3 (Table 2).

The pairwise IOD ratios for band 2/band 1 ($F_{[7,261]}$ = 4.23, $P < 0.001$), band 3/band 1 ($F_{[7,261]}$ = 15.61, $P < 0.001$), and band 3/band 2 ($F_{[7,261]}$ = 3.649, $P < 0.001$) all varied significantly among populations. For all three ratios, mean values were nonoverlapping between all five west coast Vancouver Island populations and the Big Qualicum and fish farm populations (Table 2).

Population Structure

Hierarchial cluster analysis, based on pairwise distance measurements between populations, depicted a close relationship among the two fish farm and the Big Qualicum populations, and a great distinction between those populations and all of the west coast of Vancouver Island populations (Fig. 4). Among the west coast populations, the Nitinat, Conuma, and Kennedy populations were very similar, and

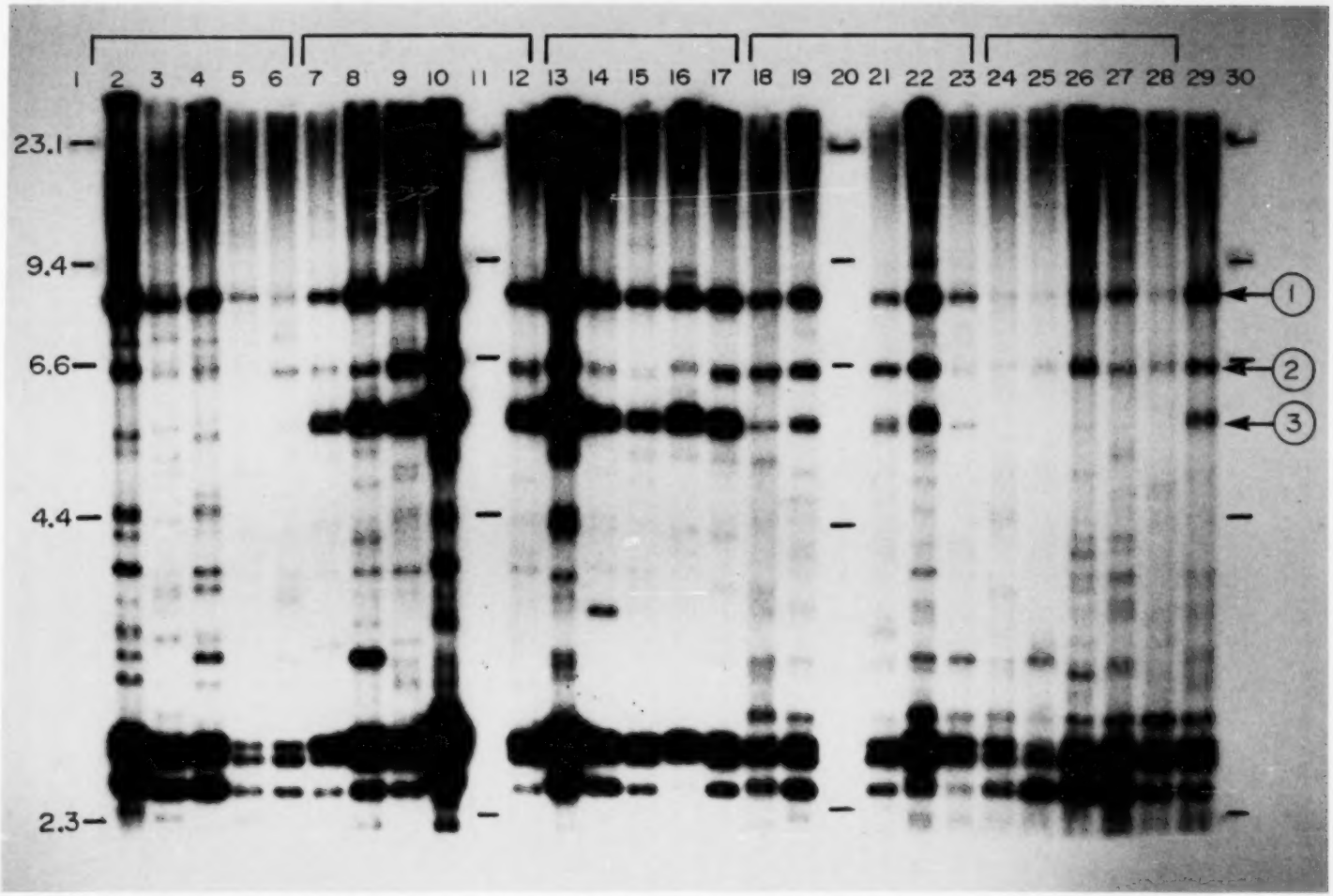

FIG. 3. Southern blot of *Bam*HI-digested DNA from farm-reared and native chinook salmon probed with B2-2. Lanes 1, 11, 20, and 30 contain λ DNA size markers. Lanes 2–6 contain Kennedy River DNA samples, lanes 7–10 and 12 contain fish farm 2 DNA samples, lanes 13–17 contain Big Qualicum River DNA samples, lanes 18, 19, and 21–23 contain Robertson Creek DNA samples, lanes 24–28 contain Conuma River DNA samples, and lane 29 contains DNA from the standard fish Robertson Creek 18. Intensity variation in the 2.60 and 2.65-kbp doublet indicates variability in DNA loading. Polymorphism in band presence and absence, and intensity, occurs at 8.3 kbp (band 1), 6.5 kbp (band 2), and 5.6 kbp (band 3).

TABLE 1. The frequency of occurrence of the 8.3-kbp (band 1), 6.4-kbp (band 2), and 5.6-kbp (band 3) DNA fragments that hybridize to the B2-2 probe in eight chinook salmon populations. The mean integrated optical densities (IODs) calculated for those fish that possess each band, and pairwise ratios of band IODs for those fish that possess both bands of each pair, are also given. Standard errors are in parentheses.

		Band frequency			Band IOD			IOD ratios		
Population	*N*	1	2	3	1	2	3	Bands 2/1	Bands 3/1	Bands 3/2
Robertson	26	1.00	1.00	0.96	9.80 (1.10)	7.63 (0.96)	8.83 (1.08)	0.89 (0.10)	1.03 (0.15)	1.50 (0.22)
Thornton	37	0.95	0.86	0.97	8.67 (0.87)	6.38 (0.67)	9.17 (1.08)	0.80 (0.09)	1.13 (0.12)	1.80 (0.22)
Conuma	28	1.00	0.92	0.32	5.48 (0.75)	3.55 (0.43)	1.34 (0.46)	0.84 (0.09)	0.27 (0.06)	0.49 (0.09)
Kennedy	49	0.96	0.67	0.29	7.89 (0.98)	4.16 (0.69)	3.94 (1.41)	0.76 (0.15)	0.51 (0.08)	1.62 (0.55)
Nitinat	27	0.96	0.56	0.37	3.46 (0.48)	3.33 (0.63)	1.43 (0.43)	0.92 (0.11)	0.53 (0.15)	0.45 (0.12)
Big Qualicum	37	1.00	0.68	1.00	16.44 (1.23)	6.03 (1.08)	21.71 (1.34)	0.32 (0.04)	1.54 (0.14)	4.66 (0.49)
Fish farm 2	42	1.00	0.74	1.00	11.61 (0.88)	6.07 (0.82)	16.60 (0.93)	0.44 (0.05)	1.62 (0.14)	4.03 (0.42)
Fish farm 1	23	1.00	0.78	1.00	6.50 (0.97)	3.64 (0.93)	10.45 (1.62)	0.52 (0.09)	1.98 (0.42)	3.25 (0.59)

TABLE 2. The mean integrated optical densities (IODs) and pairwise IOD ratios for the 8.3-kbp (band 1), 6.4-kbp (band 2), and 5.6-kbp (band 3) DNA fragments that hybridize to the B2-2 probe, calculated over all fish in each of eight chinook salmon populations. Fish missing a particular band (i.e., IOD = 0) were included in the estimation of the population mean. The mean values of the proportion of total lane IOD contributed by the sum of the band 1, 2, and 3 IODs is also given. Standard errors are in parentheses.

	Band IOD			IOD ratios			
Population	1	2	3	Bands 2/1	Bands 3/1	Bands 3/2	Band sum IOD
Robertson	9.80 (1.10)	7.63 (0.96)	8.50 (1.09)	0.89 (0.10)	0.99 (0.15)	1.44 (0.22)	0.35 (0.02)
Thornton	8.44 (0.87)	5.52 (0.70)	8.92 (1.08)	0.96 (0.18)	1.31 (0.19)	2.24 (0.26)	0.30 (0.02)
Conuma	5.48 (0.75)	3.17 (0.44)	0.43 (0.19)	0.75 (0.09)	0.09 (0.03)	0.69 (0.29)	0.16 (0.02)
Kennedy	7.57 (0.97)	2.89 (0.55)	1.13 (0.49)	0.72 (0.17)	0.35 (0.14)	1.89 (0.33)	0.22 (0.02)
Nitinat	3.33 (0.49)	1.85 (0.47)	0.53 (0.20)	0.69 (0.20)	0.38 (0.19)	2.34 (0.47)	0.17 (0.03)
Big Qualicum	16.44 (1.23)	4.08 (0.87)	21.71 (1.34)	0.21 (0.04)	1.55 (0.13)	6.69 (1.77)	0.46 (0.01)
Fish farm 2	11.61 (0.88)	4.48 (0.73)	16.60 (0.93)	0.33 (0.05)	1.70 (0.90)	5.96 (1.03)	0.41 (0.02)
Fish farm 1	6.50 (0.97)	2.85 (0.79)	10.45 (1.62)	0.41 (0.08)	1.90 (0.35)	5.37 (0.74)	0.34 (0.02)

differentiated from the Robertson and Thornton populations (Fig. 4).

Stepwise discriminant analysis on the eight populations using the seven IOD variables (band 1, 2, and 3 IODs; bands 2/1, 3/1, and 3/2 IOD ratios; and band 1 + 2 + 3 IOD/lane IOD) revealed that the IOD of band 3, the 5.6-kbp DNA fragment, was the most important variable in stock discrimination ($F_{[7,255]} = 71.19$, $P < 0.001$). The IOD values of bands 2 and 1, the proportion of lane IOD accounted for by the sum of the band 1, 2, and 3 IODs, and the IOD ratios of bands 3/1 and bands 2/1, in that order, were all significantly different among populations (all $P < 0.001$). Only the band 3/band 2 IOD ratio failed to increase stock discrimination significantly ($P > 0.05$).

Given the distinction of banding patterns between the west coast chinook salmon populations, and the fish farm and Big Qualicum populations (Fig. 4), and our interest in differentiating escaped farm fish from native fish in west coast spawning streams, we excluded the data for Big Qualicum fish and conducted a second stepwise discriminant analysis on native (Nitinat, Kennedy, Conuma, Robertson, and Thornton) and cultured (fish farms 1 and 2) chinook salmon from the west coast of Vancouver Island. Again, the band 3 IOD provided the most discrimination between the two groups ($F_{[1,226]} = 138.90$, $P < 0.001$). The band 2 IOD and the band 3/band 2 IOD ratio, in that order, significantly increased the discrimination of native and farmed fish (both $P < 0.01$).

Use of discriminant analysis or neural network analysis provided accurate classification of the test fish that were not used in development of the discriminant function or in training the network. For both methods, the average level of correct classification of wild fish was greater than 95%, and that of farm-reared fish was greater than 80% (Table 3). Thus, there was no difference in accuracy between the two methods of classification. The great majority of incorrectly classified farm fish was from farm 1, for which there was a smaller sample size (23 fish).

Discussion

The genetic impact of successful spawning of escaped farm-reared salmonids on local populations depends on the degree of genetic differentiation between the native and farm fish, and the relative numbers of the two types of fish present on local spawning grounds. Large-scale aquaculture of salmonid fishes in the marine environment is now conducted worldwide and, in some regions, escaped farm fish outnumber naturally produced fish in spawning streams (Gausen and Moen 1991). There is ample evidence that under such circumstances the population size and structure of the native fish can be affected detrimentally through interbreeding and competition with the cultured fish (Hindar et al. 1991). There are numerous small chinook salmon populations (less than 500 spawners) on the west coast of

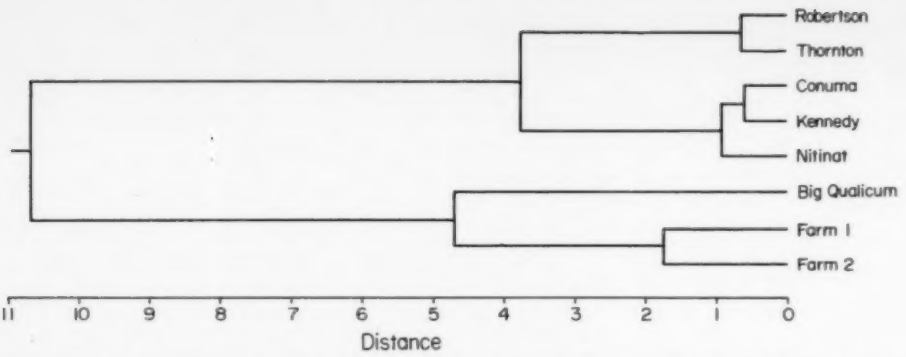

FIG. 4. Dendrogram illustrating relationships based on the presence or absence, and integrated optical densities, of the 5.6-, 6.5-, and 8.3-kbp B2-2 hybridization fragments among one Vancouver Island east coast (Big Qualicum River) and five west coast (Robertson Creek, Thornton Creek, Conuma River, Nitinat River, and Kennedy River) chinook salmon populations, and two domesticated broodstocks from fish farms located on the west coast of Vancouver Island. The fish farm populations were derived predominantly from the Big Qualicum population, and the Thornton Creek population was derived entirely from the Robertson Creek population, within the last 15 years.

TABLE 3. Percentage of correct classification of test chinook salmon DNA patterns by discriminant analysis and neural network analysis. For each trial, an independent sample of 46 (20%) of the farm and native west coast Vancouver Island DNA patterns combined was excluded from the baseline data and was used as a test sample for identification by both methods. Standard deviations are in parentheses.

	Discriminant analysis		Neural network	
Trial	Native	Farm	Native	Farm
1	96.9	92.9	93.8	100.0
2	93.6	80.0	100.0	66.7
3	97.1	83.3	97.8	83.3
4	97.1	83.3	97.1	83.3
5	97.1	72.7	97.1	81.8
Mean	96.4 (1.5)	82.4 (7.3)	97.2 (2.2)	83.0 (11.8)

Vancouver Island. Some, such as those entering Clayoquot Sound, are in close proximity to salmon farms rearing large numbers of domesticated chinook salmon. The two farm sites from which fish were sampled for the current study, and other farms in the Clayoquot Sound area, have experienced total losses of more than 50 000 large (≥1.0 kg) chinook salmon since 1990. Thus, the possibility of displacement or disruption of small local populations of chinook salmon by culture escapees is real.

The DNA banding pattern distinctions described in this study provide a means of detecting escaped farm-reared fish of Big Qualicum origin intermingling with wild fish on west coast Vancouver Island spawning grounds. The distinctive patterns that result from hybridization to the B2-2 probe result from genetic differentiation among populations and show stability over brood years (R.E. Withler and T.D. Beacham, unpublished data). They are in sharp contrast to the pattern of B2-2 hybridization on *Hae*III-restricted chinook salmon DNA, which provides more typical multilocus fingerprint patterns that can be used to differentiate even closely related individuals within populations (Stevens et al. 1993). The genetic basis of the population differentiation revealed by B2-2 on *Bam*HI-restricted DNA is not known. The multiple binding sites of B2-2 may result from multilocus VNTR variability, a class of transposable elements, or some other highly repetitive DNA sequences. The variability in band intensities of the 5.6-, 6.5- and 8.3-kbp fragments apparently reflects differences in the number of fragments of slightly different sizes as well as copy number variation for each of the fragments. Differences among fragments in the degree of homology to the B2-2 probe may also account for some of the variation in band intensity. The nucleotide sequence of B2-2 itself shows some homology to telomeric DNA repeats (Moyzis et al. 1988), providing the possibility that the variable patterns arise from variation in telomere sequences or copy number (R.E. Withler, T.D. Beacham, T.A. Stevens, unpublished data). We are currently investigating the nature of the B2-2 banding pattern in F_1 interpopulation hybrids.

It is important to note that the distinction in B2-2 patterns between farm and native chinook salmon on the west coast of Vancouver Island is apparently entirely the result of the predominantly Big Qualicum heritage of the domesticated broodstocks. It does not reflect genetic differentiation of farm fish resulting from the domestication process, nor does it provide a calibrated measure of genetic distance between the native and farm fish that can be used to predict the genetic impact, if any, that will occur as the result of the presence of farm fish in local populations. Currently, the differentiation simply provides a tool to detect and, perhaps, monitor the effects of escaped farm-reared fish in west coast waters. The use of B2-2 on *Hae*III-digested DNA to provide multilocus fingerprinting data (Stevens et al. 1993) and the use of single-locus DNA probes, such as those developed by Taggart and Ferguson (1990), may provide additional information on the impact of founder effects, genetic drift, and selection on genetic variation in the domesticated stocks. A broader genetic characterization of the farm and native populations may improve our ability to predict the consequences of intermingling.

The number of west coast Vancouver Island chinook salmon populations sampled in this study is small, and needs to be increased to ensure adequate characterization of the B2-2 variability exhibited by these populations. Nevertheless, there is no reason to believe that the populations sampled are other than representative. The most distinctive west coast population is Robertson Creek in the Somass River drainage

at the head of Barkley Inlet. This is the only population in British Columbia (of 40 thus far surveyed) not located on southeastern Vancouver Island that typically displays a B2-2 hybridization pattern in which all three of the 5.6-, 6.5-, and 8.3-kbp bands are consistently present at relatively equivalent intensities. The Thornton Creek chinook salmon population was established in a stream barren of chinook salmon through releases of Robertson Creek fish from a fish culture facility established on Thornton Creek. For brood years 1982–1985, inclusive, Robertson Creek juvenile salmon were reared in the Thornton Creek facility, and released in Thornton Creek. For brood years since 1985, fish released in Thornton Creek have been progeny of adult chinook salmon that returned to the creek, presumably from the earlier releases of Robertson Creek fish. The DNA samples from Thornton Creek fish examined in this study came from adults that returned to Thornton Creek in 1992. Like the farm-reared chinook salmon that retain B2-2 patterns similar to those of the Big Qualicum population from which they were derived within the past dozen years, the Thornton Creek chinook salmon possess B2-2 patterns clearly reflecting their recent Robertson Creek derivation.

In this analysis, we wished to identify individual fish as farm-reared or native in origin to determine the feasibility of selective removal of farm-reared fish from wild spawning areas. We chose to apply discriminant analysis, a standard statistical technique, and also neural network analysis to the problem of classifying individuals to groups. Neural networks have been applied to biological problems involving pattern recognition (Demeler and Zhou 1991; Weinstein et al. 1992) and, in some cases, have provided more accurate estimates than linear discriminant analysis (Weinstein et al. 1992). In our application, both analyses produced similar levels of accuracy of classification of new individuals. On average, 97% of native and 83% of farm chinook salmon were classified correctly as the result of either discriminant analysis or neural network analysis of DNA patterns resulting from hybridization of the B2-2 probe to *Bam*HI-digested DNA. This level of accuracy is comparable to that achieved in estimating chinook salmon stock composition by maximum likelihood analysis of biochemical genetic data (Brodziak et al. 1992). However, the use of either discriminant or neural network analysis provides classification of individuals to groups, unlike the maximum likelihood methods commonly used in stock composition estimations. In this study, incorrectly classified farm-reared fish tended to be from the site represented by the smaller sample size. Greater accuracy of classification may have been achieved with a larger data baseline for that population.

The potentially wide range of applications of recombinant DNA technology to problems in fisheries management and aquaculture has been recognized for some time (Hallerman and Beckman 1988; Bentzen et al. 1991; Wright 1993). This study follows the pioneering work on the DNA fingerprint identification of indigenous striped bass in Gulf of Mexico drainages (Wirgin et al. 1991) and the Y chromosome specific probe for chinook salmon (Devlin et al. 1991) in realizing the potential for the application of this technology to fisheries issues.

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Distribution of Satellite DNA Sequences Isolated from Arctic Char, *Salvelinus alpinus*, in the Genus *Salvelinus*

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The distribution of satellite DNA sequences in the genus *Salvelinus* has been investigated by hybridization of four satellite DNA families isolated from Arctic char, *Salvelinus alpinus*, to the genomic DNA of *S. alpinus*, *S. fontinalis*, *S. malma*, *S. namaycush*, *S. confluentus*, and *S. leucomaenis*. The distribution of homologous *Alu*I, *Dra*I, and *Eco*RI satellite sequences suggests a close relationship between *S. alpinus*, *S. malma*, and *S. confluentus* with *S. leucomaenis* split from all the other species. *S. namaycush* has a closer relationship to the *S. alpinus–malma–confluentus* group than does *S. fontinalis*. The *Mbo*I satellite, which has potential as a marker for population differentiation in *S. alpinus*, has a species distribution suggestive of an ancient salmonid satellite that may have been lost from some species during transposition events.

La répartition des séquences d'ADN satellites dans le genre *Salvelinus* a été étudiée par hybridation de quatre familles d'ADN satellites isolées de l'omble chevalier, *Salvelinus alpinus*, à l'ADN génomique de *S. alpinus*, *S. fontinalis*, *S. malma*, *S. namaycush*, *S. confluentus* et *S. leucomaenis*. La distribution des séquences satellites homologues *Alu*I, *Dra*I et *Eco*RI indique une étroite relation entre *S. alpinus*, *S. malma* et *S. confluentus*; *S. leucomaenis* est séparé de toutes les autres espèces. *S. namaycush* avait une relation plus étroite avec le groupe *S. alpinus–malma–confluentus* que *S. fontinalis*. Le satellite *Mbo*I, qui offre du potentiel comme marqueur de populations chez *S. alpinus*, possède une distribution spécifique indicative d'un ancien satellite de salmonidé qui aurait pu avoir été perdu par une espèce quelconque au cours d'événements de transposition.

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Satellite DNA sequences are amongst the fastest evolving DNA sequences (Brutlag 1980) and may account for up to 66% of the nuclear genome (Skinner 1977). In most fish species, satellite DNAs are cryptic, i.e., are not separable from the main band DNA by isopycnic-gradient centrifugation (Hudson et al. 1980), although Pizon et al. (1984) used this technique to identify satellite DNA in the tetraodontid fish, *Arothron diadematus*. An alternative technique for identifying satellite DNA sequences is restriction-enzyme digestion of nuclear DNA followed by electrophoresis through agarose or acrylamide gels and ethidium-bromide staining, when satellite sequences show up as a distinct band against a background smear (Cooke 1976; Manuelidis 1976). In fish, a number of satellite DNAs have been identified and characterized with this method in the cyprinid, *Notropis lutrensis* (Moyer et al. 1988), the common carp, *Cyprinus carpio* (Datta et al. 1988), tilapia, *Oreochromis mossambicus/hornorum* (Wright 1989; Franck et al. 1992), pollock, *Pollachius virens* (Denovan and Wright 1990), and zebrafish, *Brachydanio rerio* (He et al. 1992). In the salmonids, Ferreiro et al. (1989) reported that rainbow trout, *Oncorhynchus mykiss*, DNA contained a number of repeated sequences but gave no details of their organization, Goodier and Davidson (1993) have characterized a repetitive element from Atlantic salmon, *Salmo salar*, and Hartley and Davidson (1994) have characterized a number of satellite DNA families from Arctic char, *Salvelinus alpinus*.

Although some satellite DNAs may have limited utility as taxonomic markers for anciently diverged species because of their high evolutionary rate and species specificity (Bachmann et al. 1993), they have proved useful where the sequences are highly conserved ones such as those found in urodele amphibians (Varley et al. 1990; Batistoni et al. 1991) and cichlid fishes (Franck and Wright 1993). Additionally they may provide sources of variation at the population level (Turner et al. 1991). Relationships within the salmonid fish genus *Salvelinus* are complex and although most studies agree a close relationship between Arctic char, *S. alpinus*, and Dolly Varden, *S. malma*, the position of other species is still in dispute. In this paper we describe the distribution within the genus *Salvelinus* of homologous satellite DNA sequences isolated from Arctic char, and discuss the implications this has for phylogenetic relationships.

Materials and Methods

DNA isolated from livers, or that was a gift from Dr R.B. Phillips, was digested overnight with excess restriction enzyme. Restriction-enzyme digests separated through 2 or 3% agarose gels in Tris–borate buffer were transferred to

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TABLE 1. Distribution of repeated sequences in *Salvelinus* species detected by ethidium bromide staining and hybridization to isolated Arctic char (*S. alpinus*) satellite sequences.

	*Alu*I–*Rsa*I		*Dra*I–*Bst*EII		*Eco*RI		*Mbo*I–*Bgl*II	
	EtBr[a]	Rep[b]	EtBr	Rep	EtBr	Rep	EtBr	Rep
S. alpinus	+	+	+	+	+	+	+	+
S. fontinalis	+	+	+	—	+	—	—	—
S. malma	+	+	+	+	+	+	—	—
S. namaycush	+	+	+	—	+	+	+	+
S. confluentus	+	+	+	+	+	+	—	—
S. leucomaenis	+	—	+	—	N.D.		+	+

[a]Ethidium bromide staining
[b]The repeats are pChA2, pChDr3, pChEc2, and pChMb17.

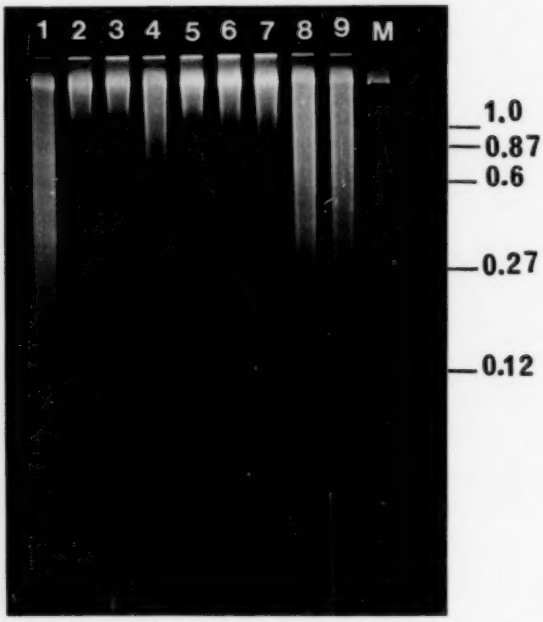

FIG. 1. Ethidium bromide stained 3% agarose gel of *S. alpinus* DNA digested with (1) *Alu*I, (2) *Bgl*II, (3) *Bst*EII, (4) *Dra*I, (5) *Eco*RI, (6) *Eco*RV, (7) *Hin*dIII, (8) *Mbo*I, and (9) *Rsa*I. Size markers (kbp) in the marker lane (M) are from λ DNA digested with *Hin*dIII and ϕX174 DNA digested with *Hae*III.

either Hybond N nylon membrane (Amersham) following denaturation in 1.5M NaCl – 0.5M NaOH using 1.5M NaCl – 0.25M NaOH as the transfer buffer, or to Biodyne B membrane (Pall Process Filtration Ltd.) following denaturation in 1.5M NaCl – 0.5M NaOH using 20× SSC (3.0M NaCl – 0.3M trisodium citrate) as the transfer buffer. Filters were prehybridized, hybridized with radiolabelled probes, and washed as in Hartley and Davidson (1994). Details of the isolation; cloning into the multifunctional phagemid pTZ18R (Pharmacia); and sequences and accession numbers of the clones pChA2, pChEc2, pChDr3, and pChMb17 containing satellite sequences from Arctic char, which were used as probes, may be found in Hartley and Davidson (1994).

Results

Nine restriction enzymes reveal repeated or satellite DNA sequences in Arctic char DNA (Fig. 1) ranging in size from approximately 100 base pairs (bp) to 1.5 kilobase pairs (kbp). Of these the *Alu*I and *Rsa*I repeats comprise one family, the *Dra*I (200-bp band) and *Bst*EII repeats make up a second family, the *Mbo*I and *Bgl*II repeats make a third family, and the EcoRI repeat and the 400-bp *Dra*I band make up a fourth family, all of which are unrelated to one another (Hartley and Davidson 1994). Ethidium bromide staining of restriction enzyme digested DNA from the six *Salvelinus* species (*S. alpinus*, *S. fontinalis*, *S. malma*, *S. namaycush*, *S. confluentus*, and *S. leucomaenis*) reveals *Alu*I (Fig. 2A), *Eco*RI (Fig. 3A), and *Dra*I (Fig. 4A) repeats in all of them (Table 1). However, when Southern blots are probed with clones representative of the *S. alpinus* satellite sequences different patterns of homology are revealed (Table 1). Clone pChA2, representative of the *Alu*I – *Rsa*I family, hybridizes to the *Alu*I satellite bands in all species except *S. leucomaenis* (Fig. 2B). Clone pChEc2, representative of the *Eco*RI – *Dra*I family hydridizes strongly to the *Eco*RI bands in *S. alpinus*, *S. malma*, and *S. confluentus*; weakly to the band in *S. namaycush*; and not at all to the band in *S. fontinalis*. There is also some hybridization to related sequences of higher molecular weight in all five species (Fig. 3B) (*S. leucomaenis* was not probed). Clone pChDr3, representative of the *Dra*I – *Bst*EII family, hybridizes to *Dra*I bands in *S. alpinus*, *S. malma*, and *S. confluentus* but not to those in *S. fontinalis*, *S. namaycush*, and *S. leucomaenis* (Fig. 4B).

The restriction enzyme *Mbo*I detects repeated sequences in *S. alpinus*, *S. namaycush*, and *S. leucomaenis* (Fig. 5A), all of which hybridize to the clone pChMb17 (Fig. 5B), which is representative of the *Mbo*I – *Bgl*II family.

Discussion

Phylogenetic relationships within *Salvelinus* are complex. Behnke (1980, 1984) recognized three subgenera, *Baione* and *Cristivomer* each with one species (*S. fontinalis* and *S. namaycush*, respectively) and *Salvelinus* containing all the other species in two species complexes. The Dolly Varden complex contained *S. malma*, *S. confluentus*, and *S. leucomaenis* and the Arctic char complex contained all the subspecies of *S. alpinus* with three different origins in Europe, Siberia, and Chukotsk Sea – Bering Sea region of the Arctic Ocean. Savvaitova (1980) recognized two groups, one of ancient origin containing *S. fontinalis*, *S. namaycush*, and *S. leucomaenis* and a more recent one including *S. alpinus* and *S. malma*. Restriction-enzyme analysis of mitochondrial DNA (Grewe et al. 1990) readily separated the three

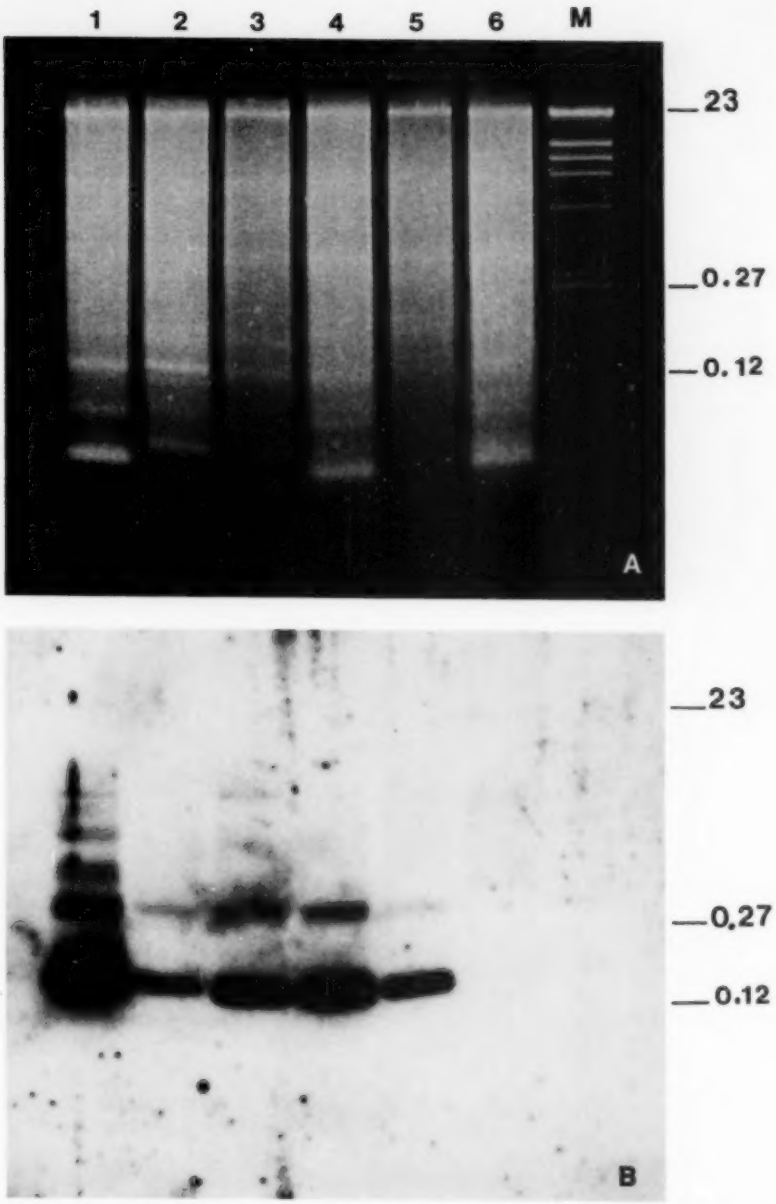

FIG. 2. (A) Ethidium bromide stained 3% agarose gel of *Alu*I-digested DNA from (1) *S. alpinus*, (2) *S. fontinalis*, (3) *S. malma*, (4) *S. namaycush*, (5) *S. confluentus*, and (6) *S. leucomaenis*. Marker lane is as in Fig. 1. (B) Southern blot of gel shown in (A) probed with pChA2.

subgenera of Behnke and distinguished two groups in *Salvelinus*: *S. malma* with two subspecies of *S. alpinus* (*S. a. alpinus* and *S. a. oquassa*) and *S. confluentus* with a third *S. alpinus* subspecies (*S. a. stagnalis*). Cytogenetic studies also group *S. malma* with *S. alpinus*, both of which have a distinct pair of telocentric chromosomes and put *S. confluentus*, with its derived karyotype, as a sister to these two (Cavender 1984). Patterns of nucleolar organizer region (NOR) differences also group *S. malma* with *S. alpinus* (Phillips et al. 1989) but group *S. confluentus* with *S. leucomaenis* (Cavender and Kimura 1989) although *S. leucomaenis* shares the same chromosome ($2n$) and chromosome arm number (NF) as *S. fontinalis* and *S. namaycush* ($2n = 84$, NF = 100). Studies utilizing restriction-enzyme analysis and sequencing of ribosomal DNA (Phillips et al. 1992; Pleyte et al. 1992) suggest three groups: *S. alpinus* with *S. malma*, *S. fontinalis* with *S. namaycush*, and *S. confluentus* with *S. leucomaenis*.

Ethidium-bromide staining of agarose gels reveals that all the *Salvelinus* species possess satellite DNA sequences.

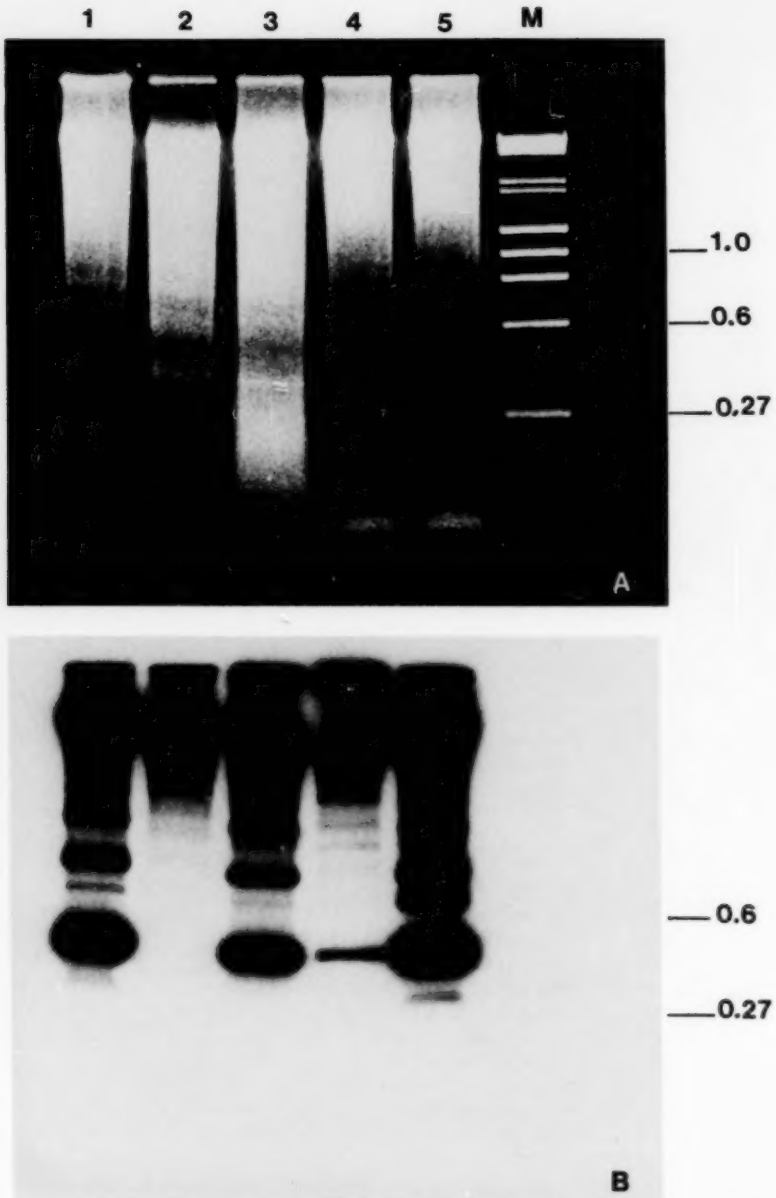

FIG. 3. (A) Ethidium bromide stained 2% agarose gel of *Eco*RI-digested DNA from (1) *S. alpinus*, (2) *S. fontinalis*, (3) *S. malma*, (4) *S. namaycush*, and (5) *S. confluentus*. Marker lane is as in Fig. 1. (B) Southern blot of the gel in (A) probed with pChEc2.

The distribution of homology to the satellite DNA sequences isolated from Arctic char provides insights into the phylogenetic relationships of the genus. The *Alu*I satellite is homologous in all species except *S. leucomaenis* and splits this species from the others. A close relationship between *S. alpinus* and *S. malma* is confirmed and *S. confluentus* is also related to this group as shown by the possession of the homologous *Dra*I satellite in these three species. The *Dra*I satellite also splits *S. namaycush* and *S. fontinalis* from this group because their *Dra*I satellites are not homologous to the *S. alpinus* one. The distribution of homologous *Eco*RI satellites splits *S. fontinalis* from the other species and groups *S. namaycush* more closely to the *S. alpinus–malma–confluentus* group. The most likely phylogenetic tree for the six *Salvelinus* species based on the distribution of these satellite DNAs is shown in Fig. 6.

The distribution of satellites related to the Arctic char *Mbo*I satellite appears to contradict the scheme shown in Fig. 6 since homologues are found in *S. namaycush* and *S. leucomaenis* but not *S. malma*, *S. confluentus*, or *S. fontinalis*. There are two possibile explanations for these observations: either the repeat has arisen recently and independently in

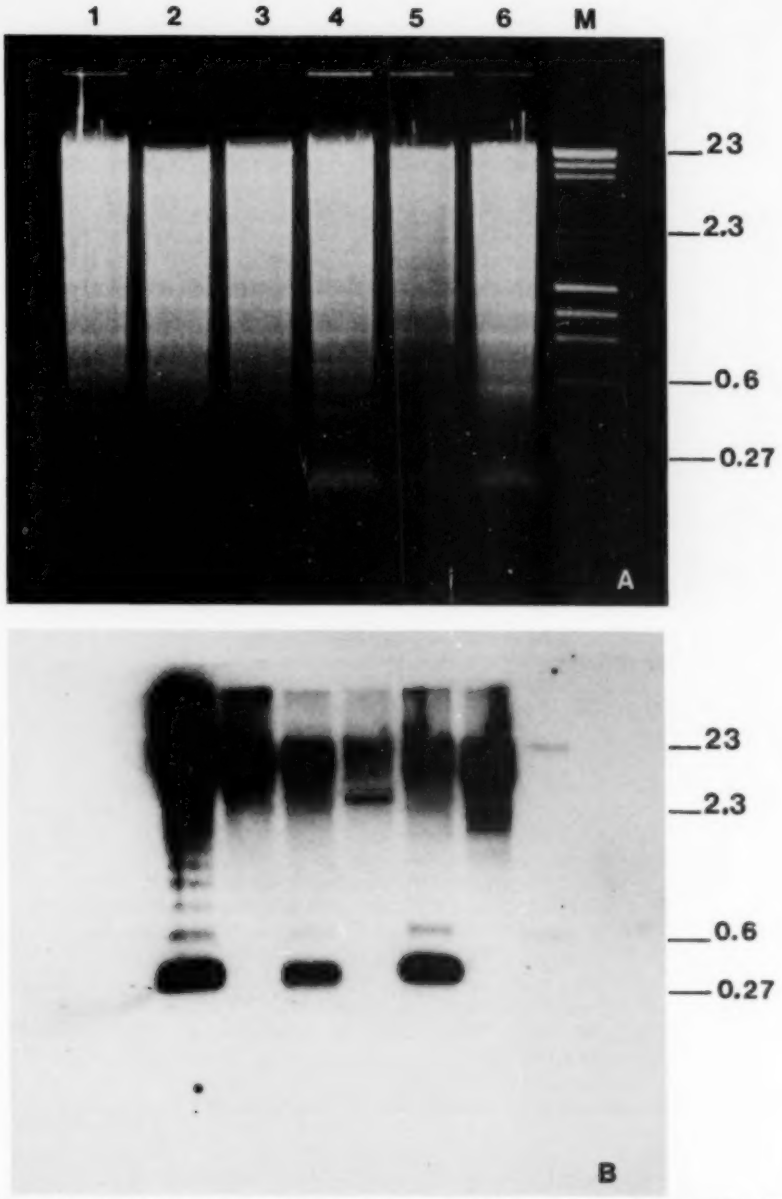

FIG. 4. (A) Ethidium bromide stained 2% agarose gel of *Dra*I-digested DNA from (1) *S. alpinus*, (2) *S. fontinalis*, (3) *S. malma*, (4) *S. namaycush*, (5) *S. confluentus*, and (6) *S. leucomaenis*. Marker lane is as in Fig. 1. (B) Southern blot of the gel in (A) probed with pChDr3.

S. alpinus, *S. namaycush*, and *S. leucomaenis* or, more likely, it is an ancient salmonid repeat that has been lost from some species over time. Several lines of evidence support the latter hypothesis. Pacific salmonids in the most recently evolved salmonid genus *Oncorhynchus* have segments of DNA that hybridize to the cloned *Mbo*I repeat in Southern blot analysis (S.E. Hartley, unpublished observation) and this suggests that the repeat was present in the common ancestor of *Salvelinus* and *Oncorhynchus*. In addition the *Mbo*I repeat has been found to be absent from an isolated population of *S. alpinus* from Wester Ross, Scotland (Hartley and Davidson 1994) but is present in all other Scottish populations examined so far (S.E. Hartley, unpublished observation).

Recent studies on repetitive elements in Atlantic salmon, *Salmo salar*, lead to a potential mechanism for the loss of the *Mbo*I repeat in other salmonid species and populations. J.L. Goodier and W.S. Davidson (unpublished observation) have identified a repeat in *Salmo salar* that has the hallmarks of a DNA-mediated transposable element like those of

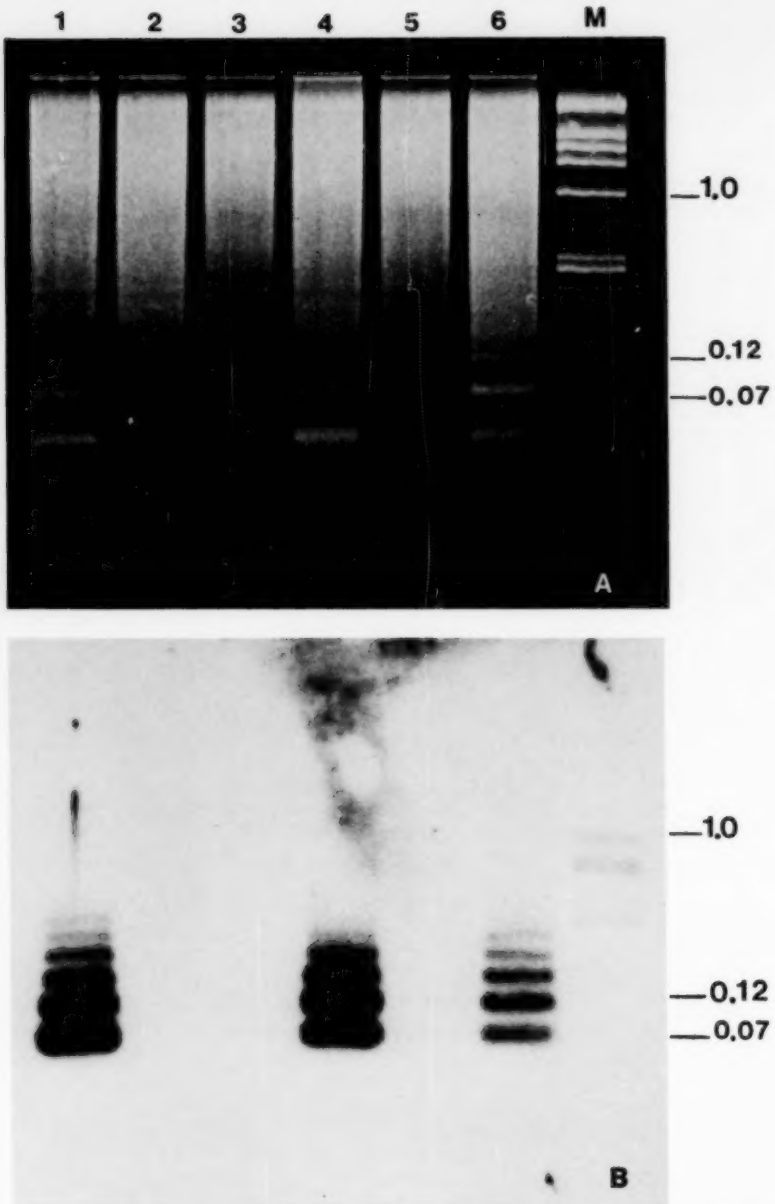

FIG. 5. (A) Ethidium bromide stained 3% agarose gel of *Mbo*I-digested DNA from (1) *S. alpinus*, (2) *S. fontinalis*, (3) *S. malma*, (4) *S. namaycush*, (5) *S. confluentus*, and (6) *S. leucomaenis*. Marker lane is as in Fig. 1. (B) Southern blot of the gel in (A) probed with pChMb17.

the nematode *C. elegans* (Rosenzweig et al. 1983). A FASTA search (Pearson 1990) of the EMBL data base with the salmon Tcl transposonlike sequence revealed that it contained a segment of DNA that was approximately 80% identical to the 5′ end of a clone (pChMb12) containing the *Mbo*I repeat from *S. alpinus*. It may be that during times of active transposition, members of the *Mbo*I repeat family are lost from salmonid genomes. The characterization of satellite DNA families from a single species provides information over and above the organization and structure of that species' genome. The distribution of the *S. alpinus* satellites in other *Salvelinus* species and in other salmonid genera, has given additional insights into the evolutionary relationships of members of the *Salvelinus* genus. This study also confirms that, as Turner et al. (1991) predicted, satellite DNAs (such as the *Mbo*I repeat) are useful as population markers within species. There is clearly a need to continue to explore all aspects and segments of the genome if we

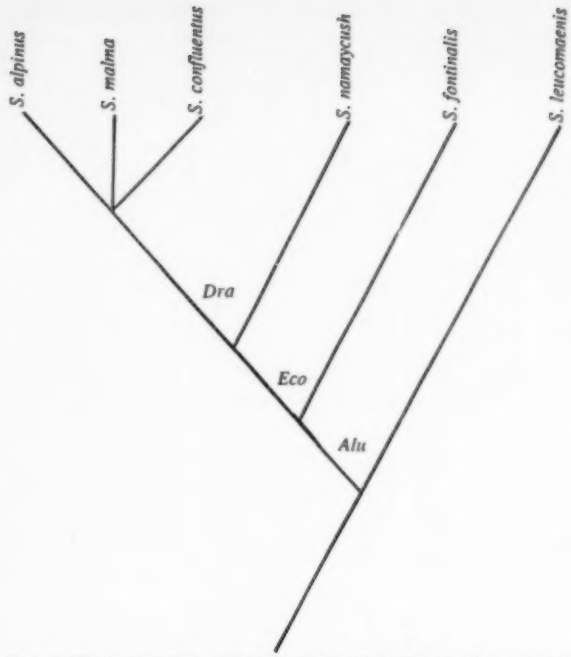

FIG. 6. Phylogenetic tree based on the distribution of sequences homologous to Arctic char satellite DNAs.

are to understand the underlying genetic mechanims of evolutionary change and population differentiation.

Acknowledgements

We are grateful to Dr R.B. Phillips for DNA samples from *S. malma*, *S. namaycush*, *S. confluentus*, and *S. leucomaenis*. This work was supported by grants from the Natural Environment Research Council of Great Britain (S.E.H), the Natural Sciences and Engineering Research Council of Canada (W.S.D.), and NATO.

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Heterogeneity in the Distribution of Mitochondrial DNA Haplotypes in Female Rainbow Trout Spawning in Different Seasons

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Danzmann, R.G., M.M. Ferguson, and D.M. Heculuck. 1994. Heterogeneity in the distribution of mitochondrial DNA haplotypes in female rainbow trout spawning in different seasons. Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 284–289.

Analysis of females spawning throughout the year (October to late February) at a commercial fish farm in Ontario, revealed significant heterogeneity in the distribution of their mitochondrial DNA (mtDNA) haplotypes. These findings are consistent with previous data from this stock and indicate that female– daughter spawning times are temporally stable. This suggests a high maternal genetic contribution to this trait. Conversely, no temporal heterogeneity in the spawning times of females with different mtDNA haplotypes was observed in an unselected government (Ontario Ministry of Natural Resources) hatchery stock recently derived from a wild naturalized population. The government hatchery stock is normally spring spawning and has much higher levels of nucleon diversity compared with the cultured stock. Therefore, it appears that only a limited number of family lines within unselected stocks may show the propensity towards greatly accelerated spawning times. Furthermore, associations between spawning time and mtDNA haplotype may only become apparent following several generations of artificial selection.

L'étude de femelles qui fraient toute l'année (d'octobre à la fin février) dans un établissement piscicole commercial en Ontario a révélé, chez des dernières, une hétérogénéité substantielle en ce qui a trait à la distribution des haplotypes d'ADN mitochondrial (ADNmt). Ces constatations convergent avec des données antérieures sur ce stock et indiquent que les périodes de frai des femelles et de leurs filles sont stables temporairement. À l'opposé, aucune hétérogénéité temporelle dans les périodes de frai des femelles ayant des haplotypes d'ADNmt différents n'a été observée dans un stock d'écloserie non sélectionné du gouvernement (ministère des ressources naturelles de l'Ontario) dérivé récemment d'une population sauvage naturalisée. Le stock de l'écloserie du gouvernement fraie normalement au printemps et possède un degré de diversité des nucléons beaucoup plus élevé que le stock d'élevage. Par conséquent, il semblerait que seul un nombre limité des lignées familiales dans des stocks non sélectionnés montre une propension à avoir des périodes de frai grandement accélérées. De plus, les relations entre période de frai et haplotype d'ADNmt peuvent ne devenir apparentes qu'après plusieurs générations de sélection artificielle.

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The conservation of genetic variation within fish stocks is one of the primary goals of fisheries management. However, in aquacultural programs the goal is often the selection of the available genetic variation to produce a desirable fish for the immediate culture conditions. This selection often leads to the loss of additive genetic variation controlling many phenotypic and production traits. Spawning time in rainbow trout (*Oncorhynchus mykiss*) is one such trait, and appears to be controlled by many loci acting additively (Siitonen and Gall 1989). Rainbow trout are historically spring spawning, but selection for earlier spawning has resulted in the establishment of fall spawning strains (Busack and Gall 1980).

Comparisons of allozyme variability among fish spawning in different parts of the spawning season also suggests that many gene loci, acting additively, control spawning time. Leary et al. (1989) reported higher allozyme heterozygosity in fish from the middle of the spawning distribution for one hatchery strain and higher levels at the beginning and middle for another strain. Similar results have been observed with hatching time distributions of rainbow trout (Danzmann and Ferguson 1988). These results are expected if variant alleles at many loci influence the trait or there is linkage disequilibrium between marker loci and loci with direct effects. Heterozygotes will be intermediate between two extreme phenotypic values by chance alone since the alternate homozygotes will more often be associated with the extremes (Chakraborty and Ryman 1983).

The environmental control of spawning time in salmonids is well established and is primarily mediated by photoperiod (Bromage et al. 1982; Bromage and Duston 1986) which induces changes in hormonal cycles (Zohar 1990). However, the genetic basis for spawn timing differences among fish that are subjected to identical environmental conditions is poorly understood. Available genetic data indicate that realized heritability estimates for spawning time are fairly high in

TABLE 1. Spawning collections of Ganaraska rainbow trout at the Normandale FCS.

1991 Date	*N*	Proportion spawned	1992 Date	*N*	Proportion spawned
January 4	16[b]	15.5	February 5	10[a] 12[b]	20[c]
February 6	16[b]	52.5	February 19	6[a] 12[b]	45
February 20	15[b]	100.0	March 4	9[a] 12[b]	75
			March 18	11[a] 10[b]	95

[a]Number of 3-yr-old females sampled.
[b]Number of 4-yr-old females sampled.
[c]Approximate estimate for both 3- and 4-year-old females combined.

rainbow trout with an estimated maximum of 0.50 (Siitonen and Gall 1989). Few studies, however, have directly examined the spawning time of daughters compared with mothers. Lane et al. (1990) and Gharrett and Smoker (1991) demonstrated that the run timing (early versus late) of pink salmon (*Oncorhynchus gorbuscha*) offspring from a number of families was most similar to that of their parents. Gall (1983) also reported that although daughters tend to have the same spawning times as their mothers, there was a tendency for daughters from late-spawning females to have faster maturation rates to spawning than daughters from early spawning females. These data suggested that spawning times of daughters in subsequent generations would tend more toward the mean spawning time of the strain. Genetic markers for spawning time may then be somewhat obscured due to the reciprocal association of mother and daughter maturation rates.

Ontario aquaculture strains of rainbow trout show nonrandom distributions of mitocondrial DNA (mtDNA) haplotypes across spawning seasons (Ferguson et al. 1992, 1993). Two haplotypes predominate in the Ontario fish with haplotype 13 having a higher frequency in the progeny of fall spawning fish compared with haplotype 10 which predominates in the progeny of spring spawning fish. At one farm (Goosens), haplotype 13 represented 94% of the progeny of fall spawning fish, 20% of winter fish, and was absent in the spring spawning group. Conversely, haplotype 10 increased in frequency from 6% in the progeny of fall fish, 27% in winter fish, and represented 100% in spring fish. Although these data suggest that Goosens females spawning in different seasons differ in mtDNA haplotype distributions, they are relatively indirect because they are based on a limited sampling of progeny and not of the females themselves. Heterogeneity in mtDNA haplotypes across seasons may have been caused by a limited number of females contributing unequal numbers of progeny to a particular spawning lot.

We performed a detailed and more direct examination of mtDNA variability in females spawning from October to February at Goosens Trout Farm by sampling their ovulated eggs. If mtDNA haplotypes are not markers of season-specific female–daughter spawning times (i.e., daughters having the same spawning time as their mothers), we would not expect any repeatable association between spawning season and the genetic mtDNA haplotype of females spawning in that season. We also examined this association in a genetically diverse hatchery stock (Danzmann et al. 1993; Ferguson et al. 1993) used for the rehabilitation of natural populations. This spring-spawning hatchery stock was founded from large numbers of individuals captured from the Ganaraska River, Ontario, and its breeding program has been designed to maintain that diversity without artificial selection (Ferguson et al. 1991). Higher frequencies of haplotypes (family lines) in either tail of the spawning distribution may represent those female lines that could be most effectively selected for increased or delayed spawning times. We anticipated that the greatest mtDNA haplotype diversity would be present in the middle of the spawning distribution based on what has been observed with the distribution allozyme variability associated with spawning and hatching times in rainbow trout (Danzmann and Ferguson 1988; Leary et al. 1989). Because mtDNA is haploid, the mechanism by which greater mtDNA variability is expected in the centre of the distribution results from the averaging effects across the tails of distributions of divergent spawning times among mtDNA family lines, rather than additive gene effects.

Materials and Methods

Fish Collections

Ovulated eggs were collected from females at bi- to triweekly intervals at Goosens Trout Farm in 1992 and 1993. The first (designated week 0) of eight collections was made October 21, 1992, and occurred 2–3 wk after the commencement of spawning. The last collection occurred on February 12, 1993, and was 1–2 wk before the cessation of spawning for Goosens spring fish.

Collections of ovulated eggs were taken from Ganaraska females at the Ontario Ministry of Natural Resources (OMNR) Normandale Fish Culture Station (FCS) throughout the spawning periods of 1991 and 1992 (late December – early January to late February – early March). The naturalized population of rainbow trout in the Ganaraska River spawns from March to early May. The acceleration of spawning times as well as other life-history variables is common in hatchery populations of salmonid fishes and results from enhanced nutrition and warmer rearing temperatures (Thorpe 1991).

Approximately 200 four-year-old Ganaraska females were spawned at the Normandale FCS. in 1991. We sampled 16 of 25 females spawning on February 6 and 16 of 50 females spawning on February 20, 1991. We were unable to sample females from the beginning of the spawning distribution in 1991. Therefore, we sampled 16 juveniles from a pool

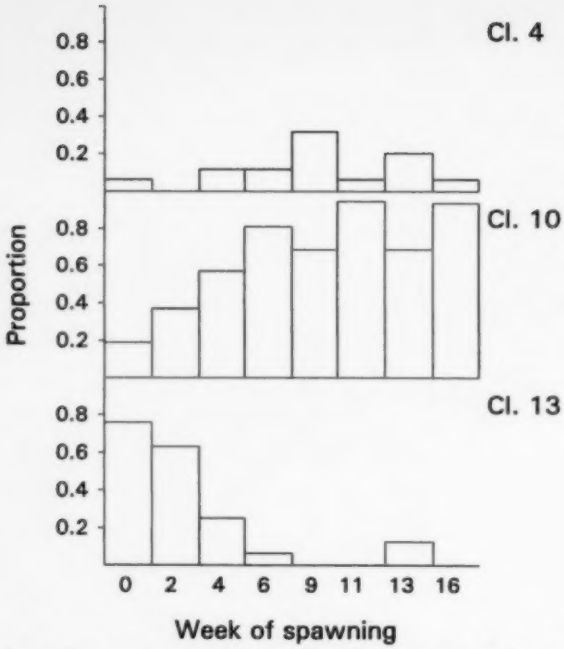

FIG. 1. The relative proportion of haplotypes 4, 10, and 13 in females from Goosens hatchery stock sampled throughout their yearly spawning cycle. Week 0 represents the first sample taken on October 21, 1992. Week 16 represents the last sample taken on February 12, 1993. A single haplotype 22 individual was sampled in week 4 (not shown). Nucleon diversities (h) among the females spawning groups starting with week 0 are as follows: 0.425; 0.495; 0.642; 0.342; 0.458; 0.133; 0.508; 0.125.

produced from combining equal numbers of progeny from 31 full-sib families (31 females) spawned on January 4. This represented all the females spawning on the first spawning collection for the season (Table 1).

In 1992, the first egg collection at the Normandale FCS was February 5 and represented the second week of spawning. Both 3- and 4-yr-old females were sampled in 1992 (Table 1). Spawning collections in 1992 were made on 2 or 3 d throughout the work week at the Normandale F.C.S. such that all the females spawning in that particular week were not sampled. The number of 3- or 4-year old females sampled on each collection day represent all the 3-yr-old females that were spawned on that date plus a maximum sample of twelve 4-yr-old females.

Mitochondrial DNA Preparation and Data Analysis

Mitochondrial DNA was isolated from ovulated eggs and livers according to the method of Chapman and Powers (1984) and as described by Danzmann et al. (1991). The partially purified mtDNA obtained with this technique was digested with 15 six-base recognition restriction enzymes (*Apa*I, *Ava*I, *Bam*HI, *Bgl*II, *Csp*45I, *Dra*II, *Eco*RV, *Hin*cII, *Hin*dIII, *Hpa*I, *Mam*I, *Nhe*I, *Sna*BI, *Ssp*I, and *Sty*I) which generate RFLPs in rainbow trout (Danzmann et al. 1993). Although *Hpa*I is an isoschizomer of *Hin*cII it was occasionally used in this study since a polymorphism detected by *Hpa*I occurs in a small *Hin*cII fragment that is not readily detected without the use of a probe. DNA fragments were visualized in 0.8, 1.0, or 1.2% horizontal agarose gels following ethidium-bromide staining. All RFLP patterns not directly visualized were detected following Southern transfer to Hybond N membranes (Amersham Corp.) and chemiluminescent detection with the Boehringer-Mannheim AMPPD-DIG system (Höltke et al. 1992). Southern transfer was performed under vacuum according to the manufacturer's specifications (Pharmacia LKB vacublotter) but without depurination. Denaturation and neutralization were conducted for one-half hour each, followed by 20× SSC transfer for 1–1.5 h. Nylon membranes were baked overnight at 80°C prior to hybridization. Random-primed (Feinberg and Vogelstein 1983) DIG-labelled mtDNA probes used in the hybridizations were obtained from mtDNA isolated from ova. Ovulated eggs are a rich source of mtDNA and contain proportionately much less nuclear DNA and RNA contamination compared with other tissues.

Many rare haplotypes were represented in Ganaraska females from each spawning collection because this strain is characterized by high nucleon diversity (Ferguson et al. 1992). This yields low power and makes it difficult to test for heterogeneity in haplotype distributions given the limited number of females we could sample. Therefore, mtDNA haplotypes were redesignated according to the major phylogenetic assemblage in which they occurred. Five major mtDNA phylogenetic assemblages have been identified in rainbow trout with a Wagner parsimony and neighbour-joining analysis (Danzmann et al. 1993), and many rare haplotypes within each lineage generally differ by only one site gain or loss from the one or two most common haplotypes in the phylogenetic assemblage. We then tested for differences in frequencies of females belonging to these groupings among spawning collections.

In the Goosens strain, only four haplotypes representing three major phylogenetic assemblages were detected. One of these haplotypes (22) was represented by a single fish and was combined with haplotype 10 from which it differs by only a single site loss.

Differences in the spawning time of females belonging to different phylogenetic assemblages was tested with a contingency χ^2-test with Yate's correction for small sample sizes (Sokal and Rohlf 1981). Nucleon diversities were calculated according to Nei and Tajima (1981).

Results

Goosens females spawning in different weeks varied significantly in mtDNA haplotype distributions (contingency $\chi^2 = 50.243$, df = 14, $P < 0.001$). Haplotype 13 females (D phylogenetic assemblage) comprised a significantly greater proportion of fall spawners at Goosens farm (Fig. 1). Nucleon diversities were higher in the winter and fall spawning fish compared to spring spawning fish at Goosens farm (Fig. 1) which was previously observed (Ferguson et al. 1993).

The haplotype distributions of 3- and 4-yr-old Ganaraska females sampled during each spawning collection at the Normandale F.C.S. in 1992 did not differ significantly (contingency χ^2, $P > 0.70$) (Table 2). Therefore, both year-classes were combined for analysis of heterogeneity in mtDNA phylogenetic assemblages among spawning collections. No significant heterogeneity in the spawning times of females from different phylogenetic assemblages was detected in

TABLE 2. The number of females with different mtDNA haplotype lineages sampled in different spawning collections at the Normandale FCS in 1991 and 1992.

		A[a]							B	C						D			E			
		1[b]	10	17	18	22	27	29	4	2	3	5	24	33	35	38	8	21	26	9	15	19	h[c]
1991																							
Collection 1[d]		—	2	—	—	—	—	—	—	2	5	—	—	—	—	1	2	—	—	4	—	—	0.852
Collection 2		2	3	—	1	—	—	—	3	—	1	—	—	—	—	—	5	—	—	1	—	—	0.858
Collection 3		—	3	1	—	1	—	1	—	—	1	2	1	—	—	—	2	—	1	1	—	1	0.952
1992																							
Collection 1	3+	2	2	—	—	—	—	—	—	—	2	—	1	—	—	—	—	—	—	3	—	—	0.867
	4+	1	1	—	—	—	2	—	1	—	1	—	—	1	—	—	1	—	—	3	1	—	0.939
Collection 2	3+	2	1	—	—	—	—	—	—	—	1	—	—	—	—	—	2	—	—	—	—	—	0.867
	4+	1	3	—	—	—	—	—	—	—	3	—	1	—	—	—	—	—	1	2	1	—	0.894
Collection 3	3+	1	3	—	—	—	—	—	1	—	—	—	2	—	—	—	—	—	—	2	—	—	0.861
	4+	2	2	—	—	—	—	—	—	—	3	—	—	—	—	—	1	—	—	4	—	—	0.833
Collection 4	3+	—	4	—	—	—	—	—	1	—	—	—	2	—	—	—	2	1	—	1	—	—	0.854
	4+	1	—	—	—	—	—	—	1	—	2	—	2	—	1	—	1	—	—	2	—	—	0.933

[a]Capital letters are assemblages. Descriptions for phylogenetic assemblages and haplotypes 1–30 are given in Danzmann et al. 1993. Haplotype 33 is identical to haplotype 2 (Danzmann et al. 1993) except that this haplotype possesses the *Mam*I — A phenotype (i.e., intermediate between haplotypes 2 and 3. Haplotype 35 is also identical to haplotype 2 except that it possesses a new *Bam*HI phenotype designated *Bam*HI — C. This phenotype has a new cut site gain to the 12.40 kb *Bam*HI — A fragment which produces two fragments that are approximately 8.10 kb and 4.1 kb in size. The 4.30 kb *Bam*HI — A fragment is common to both phenotypes. Haplotype 38 is identical to haplotype 5 except that it also possesses the *Bam*HI — C site gain. Haplotypes 31, 32, 34, 36, and 37 will be described elsewhere.

[b]Numbers are haplotypes.

[c]Nucleon diversity estimates.

[d]Collections refer to the temporal sequence of collections given in Table 1.

either 1991 (contingency $\chi^2 = 8.70$, df = 8, $P = 0.368$) or 1992 (contingency $\chi^2 = 3.73$, df = 12, $P = 0.987$) (Table 2). Nucleon diversities did not differ among any of the groups of females spawning at different times at the Normandale F.C.S in 1991 and 1992. In fact, h values were slightly higher in the tails of the distribution for the 4-yr-old fish in 1992, and increased slightly toward the end of the distribution in 1991 (Table 2).

Discussion

The temporal heterogeneity in spawning time distributions of females with different mtDNA haplotypes in the Goosens strain coupled with similar observations from other commercial fish farms in Ontario (Ferguson et al. 1992, 1993), indicates that female family lines have highly repeatable circannual spawning time distributions. If this were not the case, hatchery practices would have randomized any initial nonrandom founding events very quickly.

For example, if a large number of donor females with a different mtDNA haplotype and an overlapping spawning distribution with the resident stock were introduced, hatchery selection practices would randomize this haplotype throughout the entire stock within a few generations. Hatchery practices at Goosens Trout Farm are such that females are routinely checked for ovulation and unovulated females are combined with females yet to ovulate. Similarly, females ovulating earlier than other females in their group are moved forward into earlier ovulating lots (R. and B. Goosens, personal communication).

It is very unlikely that the mtDNA sequence variation detected in the Goosens strain encodes any protein products that directly influence the physiology of these fish to initiate spawning. A more plausible explanation is that mtDNA variation is a neutral marker of nuclear genes which influence spawning time. This statement is supported by the fact that other fall spawning populations such as the Randolph strain maintained by the NYDEC in New York state (Danzmann et al. 1993), and another commercial strain in Ontario (i.e., Blue Springs) (Ferguson et al. 1993) do not possess haplotype 13 lineages. Interestingly, we only detected haplotype 1 in fish from both these sources, suggesting they may have had similar origins. The Randolph strain is reportedly derived from the Wytheville strain (J. Mellon, Hatchery Manager, Randolph Hatchery). Furthermore, both these lineages occur in divergent phylogenetic assemblages (i.e., A and D) (Danzmann et al. 1993). Therefore, it is unlikely that mtDNA genome of haplotype 13 fish directly encodes any protein products that would influence the physiology of these fish to initiate early seasonal spawning, since fish in quite divergent mtDNA evolutionary lineages show similar spawning times.

In contrast to the findings from the Goosens strain, there is no association between female spawning time and mtDNA variation in the Ganaraska strain. This may be due to the lack of selection for spawning time in Ganaraska, a process common to commercial strains such as Goosens. Although females are sorted at Normandale F.C.S according to their time of ovulation, there is no separation of the resulting progeny lots according to the time of female spawning (i.e., all broodstock fish are subsequently mixed) (Bill Hooper, Manager, Normandale F.C.S). Although it is unknown whether this strain would respond to selection for early spawning time, it is anticipated that such selection would be successful given previous findings in rainbow trout (Siitonen and Gall 1989). Although haplotype 13 does not occur in a very high frequency in the Ganaraska strain (< 1%, R.G. Danzmann, M.M. Ferguson, and D.M. Heculuck, unpublished data), this does not mean that such selection would not be successful.

There is also no tendency for Ganaraska females with haplotype lineages closely related to haplotype 13 to spawn earlier. We would have expected haplotype 8 fish (one restriction site different from haplotype 13) to be more abundant in early spawning fish if there is a propensity for this haplotype to respond to early spawning selection pressure. We observed the highest frequency of this haplotype in the middle spawning collection in 1991, and a slightly higher frequency of haplotype 8 toward the end of the distribution in 1992 for both 3 and 4 yr olds. Also, no association with spawn timing was evident with haplotype 1. Since all major haplotype lineages appear to be equally represented in the early spawning distribution of Ganaraska fish, differences among female lines with respect to their propensity to show accelerated spawning times may not be evident until 2 or 3 generations of selection.

Although there are no differences in the nucleon diversities of Ganaraska fish sampled in different parts of the spawning distribution, fish spawning at different times are not necessarily genetically equivalent. The Goosens data show that differential associations with spawning time can exist among female lines. Therefore, to conserve genetic variability in natural populations, the entire spawning distribution must be sampled as suggested by Leary et al. (1989). Selection for any trait which differs among females from different mtDNA lineages will require that selection is started with females from a number of haplotypes.

The data from the present study suggest that female spawning times are more endogenously controlled within the female genome. If this were not so females with specific mtDNA haplotypes would have been randomly distributed throughout the spawning distribution as these females are crossed to "early" and "late" spawning males because of the broader times of fertilization capabilities in males. The spawning times of daughters from "late" males would be expected to be delayed compared to daughters from "early" males due to additive effects. Observations that daughters from haplotype 13 females spawn very early in the season do not support the additive influence of males on spawning times. Although studies are sparse, it appears as if realized heritabilities for spawn timing in rainbow trout may be fairly substantial based upon mother-daughter selection differentials (Siitonen and Gall 1989). Since comparable data for sire components do not exist it is unknown what the relative maternal and non-additive components to the heritability estimates are. We suspect they are quite large.

Future studies will need to examine the maternal genetic effects and dominance effects in the female genome with gonadal maturation. The reference to maternal genetic effects instead of maternal effects is to distinguish the fact that maternally influenced traits need not be exclusively nongenetic according to quantitative genetic analysis definitions. For example, the relative receptivity to hormonal induction (or calcium signalling) of ovarian follicular development may vary in females from different mtDNA phylogenetic lineages. Possibly, higher levels of testosterone or 17B-estradiol which regulate the female reproductive cycle

(Goetz 1983; Nagahama 1987), may be detected earlier in the cycle of certain female mtDNA lines. There are a plethora of postulates that may be forwarded and examined. If the variation in experimental results from physiological and genetic investigations are associated with different maternal mtDNA lineages, the evolutionary explanation for such variation becomes apparent.

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Differences in Genetic Diversity for Mitochondrial DNA Between Hatchery and Wild Populations of *Oncorhynchus*

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Nielsen, J.L., C. Gan, and W.K. Thomas. 1994. Differences in genetic diversity for mitochondrial DNA between hatchery and wild populations of *Oncorhynchus*. Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 290–297.

Amplification by the polymerase chain reaction and sequencing of a highly variable segment (199 base pairs) of mitochondrial DNA (mtDNA) in 491 fish from 22 streams and 9 hatcheries revealed 9 mtDNA types in steelhead trout (*Oncorhynchus mykiss*), 5 types in coho salmon (*O. kisutch*), and 6 types in chinook salmon (*O. tshawytscha*). Likelihood chi-square analysis of frequencies of mtDNA types in eight paired comparisons of hatchery and geographically proximate wild populations of *Oncorhynchus* showed significant differences in mtDNA genotypes. More mtDNA types were found, on average, in contemporary hatchery populations for each species than in geographically proximate wild stocks. Factors potentially leading to genetic differences in hatchery and wild stocks sampled in California were historic introductions of geographically divergent populations into hatchery brood stocks and lack of introgression of geographically divergent genotypes from the hatchery into wild populations.

L'amplification par réaction en chaîn préliminaire et le séquençage d'un segment hautement variable (199 paires de bases) d'ADN mitochondrial (ADNmt) de 491 poissons provenant de 22 cours d'eau et de neuf écloseries ont révélé l'existence de neuf types d'ADNmt chez la truite arc-en-ciel (*Oncorhynchus mykiss*), de cinq types chez le saumon coho (*O. kisutch*) et de six types chez le saumon chinook (*O. tshawytscha*). L'analyse de vraisemblance par le test de chi-carré de la fréquence des types d'ADNmt dans le cas de huit échantillons appariés d'*Oncorhynchus* d'élevage et de populations sauvages géographiquement proches des premiers a révélé des différences importantes au niveau des génotypes d'ADNmt. On a trouvé, en moyenne, plus de types d'ADNmt dans les populations d'élevage dans chacune des espèces que dans les populations sauvages géographiquement rapprochées. Les facteurs qui pourraient conduire à des différences génétiques dans les stocks d'élevage et sauvages échantillonnés en Californie étaient l'introduction, dans le passé, de populations géographiquement divergentes dans les stocks de reproduction des établissements piscicoles et l'absence d'introgression de génotypes géographiquement divergents provenant des établissements d'élevage dans les populations sauvages.

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Supplementation of wild salmon production by the introduction of hatchery-reared fish into the wild environment has become a popular, although controversial, management tool in efforts to prevent local stock extinction (Nickelson et al. 1986; Allendorf and Leary 1988). Often the genetic architecture of these hatchery populations is unknown and their potential impact on the genetic integrity of wild stocks depends on numerous environmental and selection factors (Waples 1991).

Supplementation of wild stocks in areas of low natural production with hatchery fish has been practiced in California for over 60 yr. The original genetic roots of these hatchery stocks are usually unknown. Subsequent additions of geographically distant stocks to hatchery populations through fish transfers have been common over time, further confusing their genetic structure. Recently, many wild California salmon stocks have declined to dangerously low levels (see Nehlsen et al. 1991). A 6-yr drought (1987–1992) has accentuated this decline in most wild *Oncorhynchus* species still spawning in the state. Because of this decline and a desire to maintain genetic diversity of locally adapted wild stocks, the impact of artificial hatchery production on wild stocks is a growing concern.

Efforts to sustain "native" salmonid production have been magnified by the federal and state listing of the Sacramento winter-run chinook salmon (*O. tshawytscha*) as a threatened species and pending petitions to list numerous other stocks throughout California. Hatchery operations for chinook, steelhead (*O. mykiss*), and coho salmon (*O. kisutch*) have increasingly emphasized native stocks by employing only local adults for artificial propagation, single-pair mating of returning adults, spawning throughout the duration of the run, and isolating temporally distinct spawning runs as unique brood stocks within the hatchery. The introduction of salmonid stocks from out of state is tightly controlled by the Department of Fish and Game to prevent disease contamination.

FIG. 1. Map showing sampling locations for *Oncorhynchus* mtDNA throughout California, 1990–93. Squares (■) represent hatchery locations and circles (●) represent sampling localities for wild fish. Stream locations include: (1) Van Duzen Hatchery; (2) Van Duzen River; (3) Van Arsdale Hatchery; (4) Middle Fork Eel River; (5) Kass Creek; (6) North Fork Noyo River; (7) South Fork Noyo River egg collecting station; (8) Warm Springs Hatchery; (9) Russian River; (10) Willow Creek; (11) Salmon Creek; (12) Lagunitas Creek; (13) upper Sacramento River; (14) Feather River Hatchery; (15) Mill Creek; (16) Nimbus Hatchery; (17) lower American River; (18) Deer Creek; (19) Merced Hatchery; (20) Redwood Creek; (21) Waddell Creek; (22) Big Creek Hatchery; (23) Big Creek; (24) Scott Creek; (25) Mill Creek; (26) Zayante Creek; (27) Santa Rosa Creek; (28) Whale Rock Hatchery; (29) Pico Grande Creek; (30) Santa Ynez River; and (31) Malibu Creek.

Fish transfers, however, continue among many California hatcheries, and hatchery fish are released in numerous streams known to harbor declining wild populations.

Until recently, few studies have looked at genetic differences between contemporary wild populations of salmon and the local hatchery stocks used to support their production. Owing to the diversity of management styles used in hatchery brood stock development, conflicting results have been recorded for isozyme differences in salmonid hatchery and wild stocks (see reviews by Allendorf and Ryman (1987), Skaala et al. (1990), and Hindar et al. (1991)). Recent studies comparing hatchery fish with locally adapted wild stocks

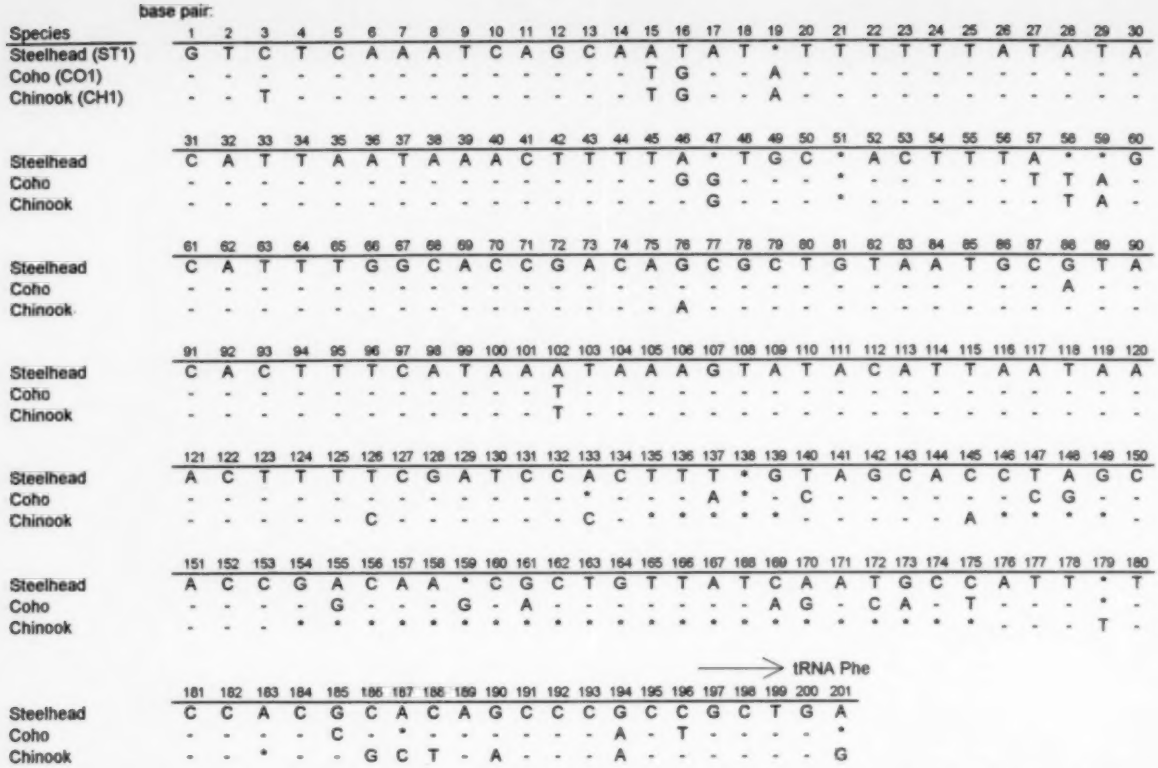

FIG. 2. Sequences (199 bp) of the highly variable 3′ end of the control region and adjacent tRNA found in the most common mtDNA type for three species of Pacific *Oncorhynchus* looked at in this study. A dash (-) denotes identity to the steelhead sequence and an asterisk (*) indicates a nucleotide deletion. The start of the phenylalanine tRNA sequence (bp 197) is indicated with an arrow. Two bp positions (Nos. 51 and 138) were absent in the three salmonid species in this study but were found in other salmon and trout mtDNAs sequenced in our laboratory. Primer S-phe is immediately adjacent to position 201. There are three bp between primer P2 and position 1.

TABLE 1. Nine variable sites and nucleotide changes found in California steelhead trout (ST), 1990–1993. An asterisk (*) represents a nucleotide deletion. The number of fish (*N*) found in this study is given for each mtDNA type. Steelhead mtDNA types ST9, ST10, ST11, and ST13 were found in steelhead populations not included in the present report. Base pair numbers follow those given in Fig. 2. Base 151 contains a cryptic (C/*) deletion that was difficult to score consistently (see Nielsen et al. 1994).

mtDNA type	*N*	Variable sites								
		65	86	94	96	130	148	151	154	194
ST1	55	T	G	T	T	T	A	A	G	G
ST2	23	C	G	T	T	T	A	A	G	G
ST3	45	T	G	T	T	T	A	A	A	G
ST4	9	T	G	T	T	C	G	A	G	G
ST5	48	T	G	T	T	C	G	C/*	G	A
ST6	10	T	G	C	T	C	G	C/*	G	A
ST7	9	T	G	T	T	C	A	A	G	A
ST8	22	T	G	T	T	C	A	C/*	G	A
ST9	0	T	G	T	T	T	A	A	G	A
ST10	0	T	G	T	C	T	A	A	A	G
ST11	0	T	A	T	T	T	A	A	G	G
ST12	9	T	G	T	T	C	A	C/*	G	G
ST13	0	T	G	T	T	C	G	C/*	G	G

have demonstrated widely divergent outcomes, such as reduced genetic diversity in hatchery stocks (Gyllensten and Wilson 1987; reviewed in Skaala et al. 1990), no detectable differences between hatchery and wild stocks in areas of intense culture (Reisenbichler and Phelps 1989; Ferguson et al. 1991), and significant differences in mitochondrial DNA (mtDNA) frequencies in cultured stocks when compared with naturalized populations (Danzmann et al. 1993). Multiple factors have been hypothesized to address these variable outcomes, including the success rate of historic stock transfers into the hatchery, small effective population size (N_e), altered selection regimes in hatchery operations, straying rates, and the direct effects of hybridization and introgression between hatchery and wild fish (Waples 1991; Hindar et al. 1991).

Molecular techniques provide new tools for the investigation of genetic diversity in salmonid populations. This study was conducted to determine if differences could be found in geographically proximate hatchery and wild populations of Pacific salmon in California, using mtDNA. The control region of the human mtDNA molecule has been shown to evolve, on average, at about 10 times the rate of the mitochondrial molecule as a whole (Greenberg et al. 1983; Kocher and Wilson 1991a), making it a prime target for intraspecific analysis (Beckenbach et al. 1990). Genotypes

TABLE 2. Ten variable sites and nucleotide changes found in coho salmon (CO) on the Noyo River, California, 1990–1992. An asterisk (*) represents a nucleotide deletion. The number of fish (*N*) is given for each mtDNA type.

mtDNA type	*N*	Variable sites									
		3	25	46	57	88	126	135	139	139a	183
CO1	46	C	T	G	T	A	T	T	G	*	A
CO2	15	C	*	G	T	A	T	T	G	*	A
CO3	2	T	T	A	A	G	C	C	G	A	A
CO4	3	C	T	G	T	G	T	T	G	*	A
CO5	7	C	T	G	T	G	T	T	G	*	G

for this maternally inherited molecule were derived from the amplification and sequencing of a highly variable section of the mtDNA control region and part of an adjacent tRNA gene, with DNA from salmonid fin tissues. Tissue from wild fish was obtained by noninvasive sampling in the field. Hatchery fish were killed at the hatchery, frozen, and shipped to the laboratory. These data indicated differing levels of genetic diversity in geographically proximate hatchery and wild Pacific salmon and trout populations in California. Hatchery populations, on the whole, showed significantly greater numbers of mtDNA types than the wild stocks within the proximate geographic area.

Materials and Methods

Salmonid tissues were collected from 31 anadromous stocks throughout California (Fig. 1) from 1990 to 1993. Salmon and trout fin tissues were taken in the field from wild adult fish during spawning migrations and at hatchery collecting weirs. Fin tissues were also obtained from juvenile fish captured during seasonal health assessments at each hatchery and during yearly population surveys of wild salmonids in California undertaken by the California Department of Fish and Game. Wild juveniles were sampled by electrofishing or seining at three to five locations throughout their natural range within each stream to prevent haploid sampling bias due to sibling aggregations.

A search of historic stocking records indicated that six populations of *Oncorhynchus* had geographically proximate populations of hatchery-based salmon and wild stocks with no record of supplementation of hatchery fish. Salmonid populations within specific drainages were considered wild if existing records indicated no direct hatchery-based introductions and if unique temporal spawning populations have been recorded for wild stocks over the last 50 yr. Biogeographic analysis of a frequency cline in steelhead mtDNA types throughout the coast of California (Nielsen 1994) supported the pooling of wild stocks of this species in broad geographic ranges. Putative wild salmonid populations were compared with geographically proximate hatchery stocks (at distances from 25 to 210 km), where salmon were cultivated to supplement wild production and released as smolts (i.e., at about 2 yr old) in adjacent stream sections or rivers.

Total genomic DNA from California salmonids was extracted from fin clips. Fin clips were taken nonintrusively from wild fish captured in the field and immediately frozen in liquid nitrogen in sterile 1.2-mL cryogenic vials. Individual fish taken at hatchery raceways and collecting weirs were frozen as whole fish on liquid nitrogen or dry ice within 2 h of collection and shipped to the laboratory where

TABLE 3. Four variable sites and nucleotide changes found in chinook salmon (CH) in California, 1991–1993 "81i" represents an 81-base exact repeat (base numbers 44–125 in Fig. 2). An asterisk (*) represents a nucleotide deletion. All chinook have 3 additional deletions found in the 3′ half of the control region. These deletions equal a total of 30 base pairs (see Fig. 2), but add no resolution to the differences found among chinook mtDNA types.

mtDNA type	*N*	Variable sites			
		47	125	134	183
CH1	73	G	T	C	*
CH2	5	A	T	C	C
CH3	17	G	T	A	*
CH4	57	G	T	C	C
CH5	34	G	T	C	A
CH6	2	G	81i	C	C

fins were clipped. DNA was extracted from a small piece of thawed fin tissue (1 mm^2) using Chelex-100 according to methods adapted by C. Orrego (University of California, Berkeley, CA) from Walsh et al. (1991). Fin tissue was submersed in 200 μL of 5% Chelex-100 resin (BioRad, catalogue no. 143-2832), placed in a 65°C water bath for 20 min, and autoclaved at 121°C for 20 min (with the autoclave set at fast exhaust). After the autoclaved samples cooled, the Chelex slurry was vortexed for 15 s and stored at 4°C or frozen until needed for polymerase chain reaction (PCR) amplification. Chelex-treated samples stored at −70°C for up to 9 mo have been amplified successfully in this study. Frozen samples were thawed in a 65°C water bath, vortexed, and then centrifuged at room temperature for 3 min at 10 000 rpm. A 2-μL aliquot of the resulting supernatant containing fish DNA was used as a template in the PCR.

Our PCR used specific primers known to amplify a highly variable segment of the mtDNA control region in salmonids (W.K. Thomas and C. Pla, unpublished data). The two primers used successfully on all salmonids were S-phe (5′-GCTTTAGTTAAGCTACG-3′) and P2 (5′-TGTTAAACC-CCTAAACCAG-3′). The resulting 3′-end segment of mtDNA control region includes part of the phenylalanine tRNA gene. Double-stranded amplifications were performed in 25-μL reactions containing 67 mM Tris-HCl (pH 8.8), 6.7 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 1 μM each of dGTP, dATP, dTTP, and dCTP, 1 μM of each

TABLE 4. Locations, number of fish (N), and mtDNA types found in geographically proximale wild and hatchery Pacific salmon populations in California, 1990–1993. Eel River wild steelhead include fish from the Middle Fork Eel River and the Van Duzen River. Chinook wild stocks include fish from Deer and Mild Creeks, the upper Sacramento River, and the lower American River. Statistical analyses are given for differences in the frequency of mtDNA types between wild and cultured population pairs using likelihood chi-square tests.

Species	Location	Stock type	N	mtDNA type (frequency in %)	Likelihood chi-square	df	p
Steelhead	Eel River	Wild	26	ST1(57), ST2(35), ST3(4), ST7(4)			
	Van Duzen Hatchery and Van Arsdale Hatchery	Cultured	22	ST1(9), ST2(23), ST3(9), ST5(4), ST7(4), ST8(14), ST12(36)	29.05	6	<0.001
	Russian R., Willow Creek, Lagunitas Cr., Redwood Cr., and Salmon Cr.	Wild	29	ST1(21), ST2(14), ST3(62), ST5(3)			
	Warm Springs Hatchery	Cultured	19	ST1(47), ST2(5), ST3(21), ST4(21), ST7(5)	18.39	5	0.002
	Scott Cr., Big Cr., Mill Cr., Waddell Cr., and Zayante Cr.	Wild	38	ST1(29), ST3(29), ST4(8), ST5(34)			
	Big Creek Hatchery	Cultured	36	ST1(22), ST2(6), ST3(25), ST4(6), ST5(22), ST7(14), ST8(6)	14.5	5	0.025
	Pico Grande Cr., Santa Ynez R., Santa Rosa Cr., and Malibu Cr.	Wild	38	ST1(5), ST2(5), ST5(61), ST6(21), ST8(8)			
	Whale Rock Hatchery	Cultured	22	ST1(9), ST5(9), St6(9), ST7(5), ST8(64), ST12(5)	33.52	6	<0.001
Coho	North Fork Noyo R., Kass Cr.	Wild	31	CO1(90), CO5(10)			
	South Fork Noyo R. egg station	Cultured	42	CO1(43), CO2(36), CO3(5), CO4(7), CO5(9)	28.39	4	<0.001
Chinook	Sacramento – San Joaquin River	Wild	50	CH1(54), CH4(34), CH5(8), CH6(4)			
	Numbus Hatchery	Cultured	52	CH1(33), CH2(6), CH3(13), CH4(31), CH5(17)	20.89	5	0.001
	Feather River Hatchery	Cultured	50	CH1(34), CH2(4), CH3(10), CH4(30), CH5(22)	18.29	5	0.003
	Merced River Hatchery	Cultured	36	CH1(33), CH3(14), CH4(25), CH5(28)	18.49	4	0.001

primer, 2 μL of the DNA extracts described above, and 0.63 units of *Thermus aquaticus* DNA polymerase (Perkin Elmer-Cetus). Amplifications were done in a Perkin Elmer-Cetus thermal cycler for 30 cycles of denaturation at 94°C for 40 s, annealing at 50°C for 1 min, and extension at 72°C for 2 min (Kocher and Wilson 1991b).

Electrophoresis of the PCR products (5 μL) containing double-stranded DNA was done on a 2% agarose gel (NuSieve, FMC) according to methods outlined in Kocher et al. (1989). The amplified product was extracted from the gel under ultraviolet light by pipeting a 5-μL plug of each sample from the center of the appropriate DNA band. This plug was melted in 200 mL of distilled water and vortexed, and 3 μL of this mixture was used as the template in a second PCR reaction to generate single-stranded DNA. Single-stranded product for sequencing was prepared by the asymmetric priming method as outlined in Kocher et al. (1989). Primer P2 was added in a 1:100 dilution relative to primer S-phe, with the rest of the amplification conditions remaining the same as in the double-stranded PCR protocol.

Control samples, containing only 23 μL of reaction mixture but no tissue extract, were included in each PCR amplification (single and double stranded) to test for reagent contamination by fish DNA. Single-stranded amplification product was desalted by washing with distilled water in Millipore microconcentrator tubes (30 000 NMWL) with three consecutive washes (300, 200, and 100 μL, respectively) and spinning the tubes in the microcentrifuge for 10 min at 4500 rpm (1800 × g) after each addition of water. Material remaining on the filter was resuspended in 20 μL of water.

The resulting purified product (3.5 μL) was subjected to direct sequencing using the primer that was limiting in the second chain reaction (P2), ^{35}SdATP and Sequenase enzyme (U.S. Biochemical) according to the manufacturer's directions except that all reactions were done in half the usual volume. The products of the sequencing reaction were separated in linear 9% polyacrylamide – 7 M urea gels, which were autoradiographed for 24–72 h at room temperature. We carried out all the DNA extractions, amplifications, and sequencing at University of California at Berkeley in 1992–1993, in the laboratory of the late A.C. Wilson (which W.K. Thomas supervised after Wilson's death in 1991).

Differences between hatchery and wild mtDNA type frequency distributions were tested for significance ($p < 0.05$) with the likelihood chi-square contingency test using the SAS statistical system release 6.07 (SAS Institute Inc., Cary, NC, USA). Differences in the frequencies of mtDNA types were tested in four paired comparisons of hatchery and wild stocks for steelhead trout from different basins in California, one comparison of hatchery and wild coho salmon, and three chinook salmon comparisons in which mtDNA frequencies found in wild fish from throughout the Sacramento – San Joaquin River system were compared with each of three hatchery populations in that basin. The number of mtDNA types found only within wild or hatchery populations were compiled for each paired comparison.

TABLE 5. The number of *Oncorhynchus* mtDNA types found only in wild or hatchery populations in paired comparisons of geographically proximate populations of anadromous salmon and trout in California, based on fish sampled from 1990 to 1993.

Species	Location	Number mtDNA types: Wild only	Number mtDNA types: Hatchery only
Steelhead	Eel River	0	3
	Russian River	1	2
	Big Creek Hatchery	0	3
	Whale Rock Hatchery	1	2
	Total steelhead	2	10
Coho	Noyo River	0	3
Chinook	Sacramento – San Joaquin R.	1	2
Overall total		3	15

Results

The conserved primers S-phe and P2 amplify approximately 207–235 base pairs (bp) of the salmonid mtDNA molecule (including the primers). The entire fragment sequenced contained 163–192 bases from the 3′ end of the control region and a 4–5 bp section of the adjacent tRNA in the three species of *Oncorhynchus* examined here (Fig. 2). Our sequenced section of the mtDNA molecule was equivalent to a segment found by Bernatchez et al. (1992; positions 103–308 in their Fig. 1) in brown trout (*Salmo trutta*), with length variations totaling 19 bp plus 37 base substitutions between our steelhead reference sequence (ST1 in Fig. 2) and the published brown trout sequence.

Bases for our salmonid mtDNA sequence were clearly readable at all positions reported here, and sequences were reread independently by a second researcher for each fish to establish sequence confirmation. The alternate strand was sequenced by limiting the S-phe primer in the second chain reaction in steelhead (ST1) to confirm the reference sequence in this species. Length variation in the sequence amplified for the different *Oncorhynchus* species was due to several deletions or insertions found within the area between these primers. This length variation was especially prominent in chinook salmon mtDNA, where up to 31 bp were absent in comparison with the other salmon sequences from this study. Two bp sites (Nos. 51 and 138) were deleted in all species used in this study, but were present in other salmon and trout mtDNAs sequenced in our laboratory. These bp positions were retained for continuity of alignment among species.

Intraspecific variation was found within all the *Oncorhynchus* species samples from California. California steelhead populations had 9 variable sites and 13 mtDNA types (Table 1). Coho salmon from the Noyo River in California varied at 10 sites, yielding 5 mtDNA types (Table 2). Despite the fact that all Noyo River hatchery stocks are currently derived from naturally returning adult spawners collected at a weir, coho mtDNA types CO2, CO3, and CO4 were found only in hatchery populations and were not represented in the wild samples. Chinook stock samples taken from the Sacramento – San Joaquin River exhibited four variable sites with three substitutions, a deletion at one bp, and insertion of one 81-bp direct repeat, resulting in six mtDNA types (Table 3). The chinook control region also included deletions up to 22 bp in length (Fig. 2), which were scattered throughout the 3′ half of the region examined. These deletions, however, were uniform throughout the chinook types and added no additional resolution to the intraspecific analysis.

TABLE 6. Documented coho introductions into the Noyo River hatchery population from geographically distant stocks, 1959–1983 (W. Jones, personal communication).

Year	Source	Number
1959	Unknown	44 520
1962	Pudding Creek	99 604
1963	Alsea River	113 120
1963	Mad River	26 352
1964	Alsea River	129 688
1966	Klaskanine River	100 033
1973	San Lorenzo River	45 340
1974	Eel River	2 500
1975	Trinity River	60 022
1981	Mad River	22 300
1983	Trinity River	20 400
Total		663 879

Comparisons of intraspecific mtDNA types in 9 hatchery and 22 geographically proximate wild stocks (Table 4) showed significant differences in the frequency of mtDNA types for geographically proximate hatchery and wild populations (likelihood chi-square tests; $p < 0.05$). Hatchery populations carried, on average, more mtDNA types in the form of rare mitochondrial lineages than did wild populations. Fifteen mtDNA types were found only in hatchery populations in the paired comparisons of geographically proximate stocks (Table 5). Only three mtDNA types were found exclusively in wild stocks in the same comparisons.

Discussion

In this study, hatchery stocks of *Oncorhynchus* were shown to carry significantly more mtDNA types than geographically proximate wild populations. These novel types of mtDNA sometimes occurred at low frequencies within a specific hatchery population (e.g., CO3 and CO4). One probable cause of this abundance of rare types in hatchery stocks

is historic stock transfers that introduced geographically divergent mtDNA lineages into the hatchery. Further evidence for this assumption can be found in a recent analysis of allozyme frequencies in the same Noyo River coho populations used in this study (Nielsen 1994). The allozyme data revealed in the hatchery stocks numerous rare alleles not found in the local wild populations. An inverse relationship was found between the number of rare alleles in wild stocks and their distance from the hatchery stocking location.

A recent survey done by Weldon Jones of the California Department of Fish and Game showed that numerous additions of geographically diverse stocks of coho salmon were made into the Noyo River hatchery in the recent past (Table 6). Although archived samples from these introduced populations could not be found for sequence comparison, mtDNA has been demonstrated to follow broad geographic frequency clines in many fish species (Avise et al. 1984; Thomas et al. 1986; Bentzen et al. 1989; González-Villaseñor and Powers 1990). It seems likely that geographically diverse coho stocks introduced into the Noyo hatchery may have contributed novel mtDNA types at the time of their introduction. There is no reason to assume that similar stock exchanges containing geographically distinct mtDNA types were not part of the history of chinook and steelhead hatcheries in California. However, reliable hatchery records for these species were not available. In all paired comparisons of hatchery and wild stocks, 15 mtDNA types were found only in the hatchery population, whereas only three mtDNA types were found in wild stocks and not in the equivalent hatchery fish (Table 5). The mechanism by which these types of mtDNA remain within the hatchery for long periods of time, and why they apparently have not introgressed in geographically proximate wild populations cannot be explained with our current data.

It is interesting to note that one rare mtDNA type found in the Sacramento River was found only in the winter-run chinook stock (mtDNA type CH6), and this mtDNA type was not found in any hatchery population. Some degree of reproductive isolation would seem to be one mechanism maintaining this type exclusively within the winter-run population. Few wild winter-run fish were available during this study ($N = 28$) due to the extremely low numbers of returning adults in 1991 and 1992. In spite of a population bottleneck due to the declining effective population size in Sacramento River winter-run chinook, this rare mtDNA type continues as part of the wild stock.

Despite the wide dispersal of hatchery-reared rainbow trout (the resident form that lives and reproduces exclusively in fresh water) throughout California since the turn of the century, the steelhead populations used as wild stocks in this mtDNA study were selected on the basis of their having no recorded hatchery introductions (Titus et al. 1995). Several of the steelhead hatcheries used in our study were small, privately run operations that maintain a policy of taking fish only from locally adapted wild stocks. During years with poor salmonid escapement (i.e., low numbers of wild spawning adults), small hatcheries, as well as larger state-run facilities, have relied on stock transfers to sustain their operations and meet their quotas for smolt releases. It is probably stock transfers, much like the case for the coho in the Noyo River, that introduced the geographically diverse mtDNA types to the populations. Increased selection for adult female spawners (who are often limiting in hatchery production), may be one mechanism that perpetuates the maternal lineages of rare mtDNA types in the hatchery populations, especially if only a small fraction of the possible spawners carrying geographically divergent mtDNAs successfully escape boodstock collection to spawn naturally in the wild.

It is important to consider multiple factors that could lead to the results in mtDNA diversity reported in this study. Each species, each race, and each stock has a unique history of transfers and wild-hatchery spawning interactions that depend on local habitat conditions, hatchery location within the watershed, brood stock management policy, and stochastic climatic conditions. These factors can obviously change throughout time for any stock. It is significant that, at this time in California at least, multiple interactions have had similar effects on the genetic diversity found in wild and hatchery populations of *Oncorhynchus*. This trend of increased numbers of mtDNA types in hatchery salmonid populations should be monitored over time to gain insight into the evolutionary significance of historic hatchery transfers and introductions. Similar data could be used to help design management options needed for the maintenance of locally adapted salmonid populations to preserve the natural biogeographic relationships and the genetic diversity found in unique populations of salmon and trout.

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Life-History Organization of Yellowstone Cutthroat trout (*Oncorhynchus clarki bouvieri*) in Yellowstone Lake

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Life-history organization of the cutthroat trout (*Oncorhynchus clarki*) may be viewed at various levels, including species, subspecies, metapopulation, population, or individual. Each level varies in spatial scale and temporal persistence, and components at each level continually change with changes in environment. Cutthroat trout are widely distributed throughout the western United States, occurring in such diverse environments as coastal rivers of the Pacific Northwest and interior streams of the Great Basin. During its evolution the species has organized into 14 subspecies with many different life-history characteristics and habitat requirements. Within subspecies, organization is equally complex. For example, life-history traits, such as average size and age, migration strategy, and migration timing, vary among individual spawning populations of Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) in tributary streams of Yellowstone Lake. Understanding the effects of human perturbations on life-history organization is critical for management of the cutthroat trout and other polytypic salmonid species. Loss of diversity at any hierarchical level jeopardizes the long-term ability of the species to adapt to changing environments, and it may also lead to increased fluctuations in abundance and yield and increase the risk of extinction.

On peut déterminer le cycle vital de la truite fardée (*Oncorhynchus clarki*) au niveau de l'espèce, de la sous-espèce, de la métapopulation, de la population ou de l'individu. Chaque niveau varie à l'échelle spatiale et temporelle, et les composantes de chaque niveau évoluent continuellement en fonction de l'environnement. La truite fardée est répandue dans l'ouest des États-Unis, habitant des milieux aussi différents que les cours d'eau côtiers du Pacifique nord-ouest que les cours d'eau intérieurs du Grand Bassin. Au cours de son évolution, l'espèce s'est divisée en 14 sous-espèces dont les caractéristiques du cycle vital et les besoins en matière d'habitat varient. Au sein des sous-espèces, l'organisation est tout aussi complexe. Par exemple, les caractéristiques du cycle vital, comme la longueur et l'âge moyens, la stratégie migratoire et le moment de la migration, varient entre les populations de géniteurs de la truite fardée de Yellowstone (*Oncorhynchus clarki bouvieri*) peuplant les cours d'eau tributaires du lac Yellowstone. La gestion de la truite fardée et d'autres espèces de salmonidés formées d'un groupe de sous-espèces dépend de la compréhension des conséquences des perturbations anthropogènes de l'organisation du cycle vital. L'appauvrissement de la biodiversité à n'importe quel niveau hiérarchique compromet la capacité à long terme de l'espèce de s'adapter à un environnement changeant, et il peut aussi mener à des fluctuations accrues de l'abondance et du rendement, en plus d'accroître le risque d'extinction.

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For several decades scientists and conservationists have focused on the preservation of biodiversity in an attempt to curb increasing rates of environmental degradation and biological extinction (e.g., Noss 1990; Erwin 1991; Soulé 1991). In many cases, however, biodiversity has been defined exclusively in terms of species richness (Erwin 1991), while ecological function and the value of intraspecific diversity have often been ignored (Sinclair 1988; Varley and Gresswell 1988; O'Brien and Mayr 1991). Failure to recognize intraspecific variability is especially acute in the case of polytypic species that occupy diverse habitats (Brown et al. 1992; Rieman and McIntyre 1993).

Because fishes exhibit greater intraspecific phenotypic variation than other taxa (Allendorf et al. 1987), recognition of this inherent variability is extremely important for maintaining biodiversity at the species level. Understanding variation at hierarchical levels below species is the focus of current research concerning the decline of anadromous salmonids. Healey (1986) reported that although individual species of Pacific salmon (*Oncorhynchus* spp.) can be characterized by certain life-history traits (e.g., age and size at maturity, fecundity, and mortality), there is extensive variation among and within populations of these species. Some researchers have suggested that life-history variation represents adaptation to spatial and temporal variation in environment (e.g., Schaffer and Elson 1975; Carl and Healey 1984; Beacham and Murray 1987), and correlations between genetic heritability and life-history traits have been observed

FIG. 1. Yellowstone Lake, Wyoming, and major tributaries.

(Carl and Healey 1984; Gharrett and Smoker 1993; Hankin et al. 1993).

A plethora of information is available about life-history variation among and within populations of anadromous salmonids (e.g., Murphy 1968; Beacham 1984; Taylor 1990), but less has been published about indigenous populations of nonanadromous forms. Jonsson and Sandlund (1979) discussed the relationship between environmental factors and life histories for isolated fluvial populations of brown trout (*Salmo trutta*) in Norway. In a large lake in Greenland, Riget et al. (1986) were able to differentiate three ecological forms of Arctic char (*Salvelinus alpinus*) using life-history characteristics. In the western United States, Cope (1957) reported life-history differences among populations of Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) in Yellowstone Lake, and genetic divergence among isolated populations of Yellowstone cutthroat trout was investigated by Bulkley (1963).

We believe that the Yellowstone cutthroat trout in Yellowstone Lake provide an example of the importance of interpopulation life-history diversity to persistence. The objective of this paper is to synthesize information concerning spatial and temporal life-history variation of the Yellowstone cutthroat trout in Yellowstone Lake. We describe the organization of individual spawning populations in the lake basin and discuss the management implications of life-history variation among populations.

Yellowstone Lake

Yellowstone Lake is located at an elevation of 2357 m (Fig. 1). Surface area is 34 108 ha, and the shoreline length is 239 km (Kaplinski 1991). Kaplinski (1991) estimated a mean depth of 48.5 m and a maximum depth of 107 m. Vegetation in the drainage basin (261 590 ha; Benson 1961) is predominantly lodgepole pine *Pinus contorta* and subalpine meadows. A total of 124 tributaries to the lake have been identified; Yellowstone cutthroat trout have been observed spawning in 68 of these streams (Jones et al. 1987).

Growing season in the lake is short; from 1951 through 1986, an average of 205 ice-free days occurred annually (R. Gresswell, unpublished data). Diatoms dominate the phytoplankton community, but during periods of thermal stratification, blooms of *Anabaena flos-aquae*, a blue-green alga, are common (Benson 1961; Garret and Knight 1973). Zooplankton (*Diaptomus shoshone*, *Daphnia schœdleri*, and *Conochilus unicornis*) are the primary food of immature Yellowstone cutthroat trout (Benson 1961). Mature cutthroat trout feed on zooplankton, two amphipods (*Gammarus lacustris* and *Hyallela azteca*), and aquatic insect larvae found in the littoral zone (Benson 1961; Jones et al. 1990).

Yellowstone cutthroat trout and longnose dace (*Rhinichthys cataractae*) are native to Yellowstone Lake (Simon 1962). Three non-native fishes, redside shiner (*Richardsonius balteatus*), lake chub (*Couesius plumbeus*), and longnose sucker

(*Catostomus catostomus*), have become established in the lake since the 1920s. An extensive fishery data base exists for cutthroat trout in Yellowstone Lake as a result of over four decades of field investigations. These data were collected primarily for monitoring the effects of angler harvest, but they also provide important information about distribution of life-history characteristics of Yellowstone cutthroat trout among spawning tributaries to the lake and changes that have occurred through time.

Pollution and land-use practices that degrade salmonid habitat in other parts of the United States have been limited in the Yellowstone Lake basin by U.S. National Park Service policies. Despite substantial habitat protection, however, Yellowstone cutthroat trout in Yellowstone Lake historically were subjected to the effects of non-native fish introductions, spawn-taking operations, commercial fishing, and intensive sport-fishery harvest (Gresswell and Varley 1988). As a result of some or all of these factors, abundance and average size and age of cutthroat trout in the Yellowstone Lake fishery declined after World War II to a low in the late 1960s. After angler harvest was reduced in the 1970s, numbers and average size increased; however, attendant effects of anthropogenic activities on life-history characteristics of cutthroat trout have not been assessed.

Life-history Organization of Cutthroat Trout: A Perspective

Life-history organization of cutthroat trout may be examined at various hierarchical levels, including species, subspecies, metapopulation, population, or individual (Fig. 2). A metapopulation is a group of local populations that are linked by movements of individuals among the populations (Hanski and Gilpin 1991). Each hierarchical level varies in spatial scale and in temporal persistence, and components of each level continually develop and evolve with changes in the level-specific environment (Warren and Liss 1980). Environment at each level is perceived as a spatial array that changes through time. Individual life-history characteristics may interpenetrate the hierarchy so that groups of local populations and metapopulations may share similar life histories.

Systematists disagree about the evolutionary history of cutthroat trout (Behnke 1992; Stearley 1992; Stearley and Smith 1993), but fossil evidence suggests that species of Pacific trout (including cutthroat trout) originated in the Miocene (Stearley and Smith 1993). Since then, cutthroat trout have diverged into 14 subspecies (Behnke 1988). Historical distribution of cutthroat trout subspecies included temperate rain forests of the Pacific Northwest, pluvial basins within the Great Basin, subalpine areas of the Rocky Mountains, and high-deserts of the Southwest. Habitats ranged from beaver ponds to large lakes (e.g., Pyramid Lake, Nevada), and from first-order tributaries to the Columbia River. Variation in life-history characteristics such as anadromy, iteroparity, and age at maturity reflect the diverse environments and selective factors affecting the evolution of the species (Gresswell 1988). Some cutthroat trout forms can be differentiated by susceptibility to angling (Dwyer 1990).

With the possible exception of the coastal cutthroat trout (*Oncorhynchus clarki clarki*), the Yellowstone cutthroat trout is more abundant and inhabits a greater geographical range than any other subspecies of cutthroat trout (Varley and Gresswell 1988). Yellowstone cutthroat trout were indigenous to the Yellowstone River drainage in Montana and Wyoming and portions of the Snake River drainage in Wyoming, Idaho, Nevada, Utah, and possibly Washington (Behnke 1988). Numerous metapopulations of Yellowstone cutthroat trout evolved unique life-history characteristics in response to environmental changes and isolation that ensued following late Pleistocene glaciation. The Yellowstone Lake metapopulation (Fig. 2b) supported an extensive hatchery operation from 1899–1957 that led to the worldwide distribution of the subspecies. The metapopulation in Heart Lake co-evolved with seven other fish species (Smith and Kendall 1921; Varley and Schullery 1983), and piscivory is common in cutthroat trout in the lake. McBride Lake is small headwater lake; individuals in this metapopulation feed primarily on zooplankton, scuds, and aquatic insects. Yellowstone cutthroat trout from McBride Lake have been used to establish feral populations in small lakes and reservoirs in Montana (McMullin and Dotson 1988). The Yellowstone River below the Lower Falls supports a fluvial metapopulation of Yellowstone cutthroat trout that has persisted despite the introduction and establishment of non-native salmonids (Clancy 1988).

Anthropogenic activities, including habitat degradation, angler harvest, and non-native species introductions, have contracted the historical range of Yellowstone cutthroat trout. Lacustrine forms remain in approximately 85% of the historical range, but only about 10% of the original fluvial habitat is still occupied by genetically unaltered populations (Varley and Gresswell 1988). Range restriction resulted in the loss of life-history variation, especially of forms that were common to large rivers at lower elevation, such as the Yellowstone River near Miles City, Montana, and the Snake River above Shoshone Falls (Varley and Gresswell 1988).

Even in a single body of water, such as Yellowstone Lake, individual spawning populations of Yellowstone cutthroat trout differ in life-history characteristics associated with spatial and temporal environmental patterns (Gresswell and Varley 1988; Fig. 2c). On an areal basis, Yellowstone Lake represents almost 80% of the remaining historic lacustrine habitat of the Yellowstone cutthroat trout. Tributaries to the lake range from the upper Yellowstone River, a sixth-order river with an 110 000-ha drainage, to unnamed first-order streams with watersheds of less than 10 ha.

Spatial Variation of Cutthroat Trout Life Histories in Yellowstone Lake

Reproductive Isolation and Homing

Sinclair (1988) suggested that any behavior enhancing the retention of population members near the source of reproduction must ultimately be adaptive because it increases the probability of successful sexual reproduction. Although complete reproductive isolation among populations of Yellowstone cutthroat trout from spawning tributaries probably does not occur in the Yellowstone Lake drainage, there is substantial evidence of homing by adult spawners. We believe that the relative fidelity of individual spawning populations to specific tributaries supports the view that the assemblage of Yellowstone cutthroat trout in Yellowstone Lake should be considered a metapopulation. Local adaptation to specific environmental conditions in individual tributaries is evident, and concomitantly, there is evidence of movement (straying) among populations. Although occupation

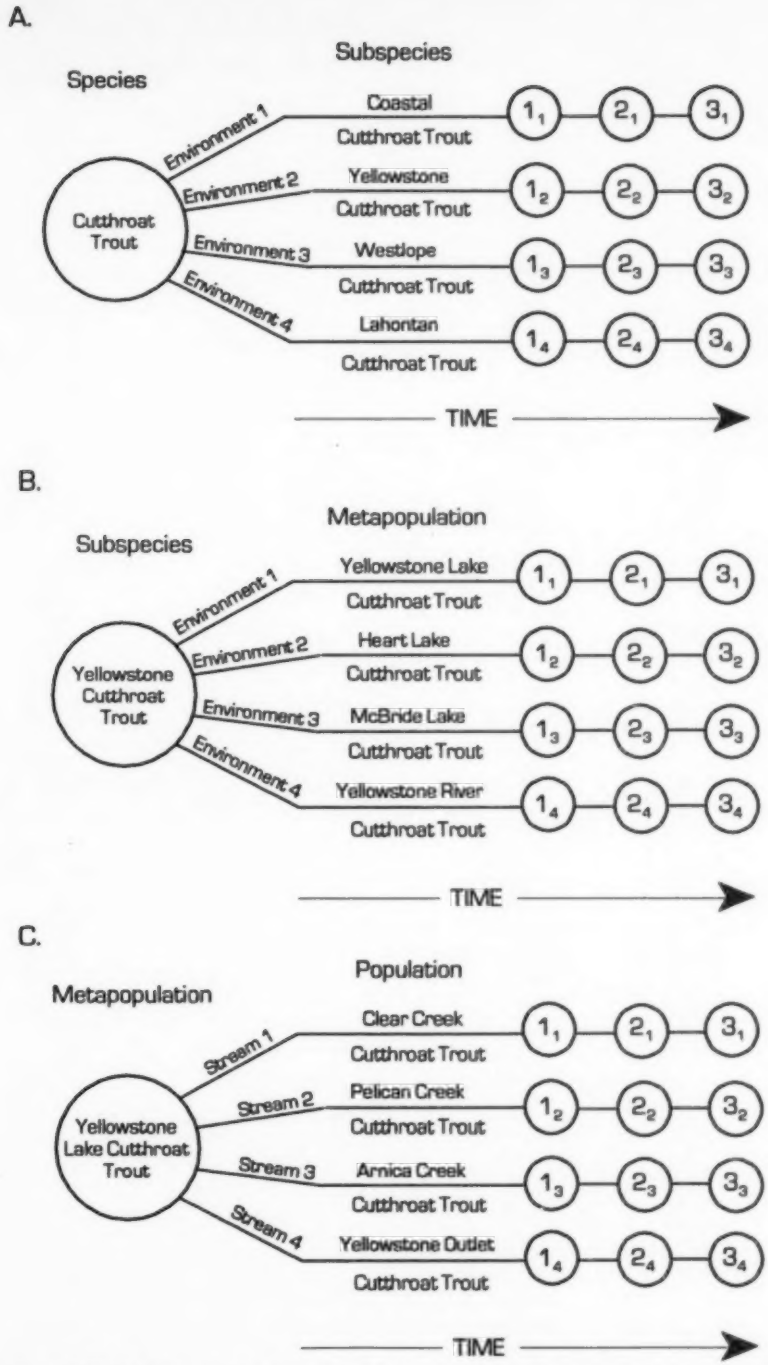

FIG. 2. A hierarchical view of life-history organization of the (a) cutthroat trout (*Oncorhynchus clarki*) species, (b) Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) subspecies, and c) Yellowstone Lake metapopulation of Yellowstone cutthroat trout. At each level in the hierarchy, subgroups adapt through time to changes in level-specific environment (adapted from Wevers 1994).

of diverse habitat patches in a spatially heterogeneous environment provides the basis for persistence individual populations, interpopulation movement is critical to the colonization of vacant habitat patches (temporal environmental heterogeneity) and, ultimately, metapopulation persistence (Hanski 1991).

Ball (1955) documented natal homing (the return of adult spawners to the area of their birth) for Yellowstone cutthroat

trout in Yellowstone Lake. Three groups of immature Yellowstone cutthroat trout were fin clipped in 1950 and 1951 as they emigrated from Arnica Creek (Fig. 1) to Yellowstone Lake. Group estimates indicated that between 16 and 25% of the clipped fish survived to return to Arnica Creek as spawners, and none of the fish marked in Arnica Creek were recovered in five other lake tributaries being monitored between 1950 and 1954.

Repeat homing behavior (individual spawners returning to the same tributary in successive years; McCleave 1967) has been observed for spawners from Yellowstone Lake. Between 1949 and 1955, 18 836 Yellowstone cutthroat trout spawners were tagged in Arnica, Chipmunk, Clear, Grouse, and Pelican creeks (Cope 1957). Approximately 97% of 244 adults returning as repeat spawners were collected in the same stream in which they were originally marked. In a more recent study, at least 23% of 42 229 spawners marked at Clear Creek in 1979 returned to spawn again between 1980 and 1984; however, an average of about 1% of the marked fish were collected annually in Pelican Creek and Cub Creek (Jones et al. 1985).

Between 1949 and 1953, the proportion of repeat spawners that returned in consecutive years or alternate years varied among streams (Ball and Cope 1961). Based on the return of fish from individual tag groups, consecutive-year spawners were most abundant in Arnica Creek and Chipmunk Creek. The proportion of consecutive- and alternative-year spawners in Pelican Creek and Grouse Creek varied among years and tag groups. At Clear Creek, however, spawners marked in 1979 returned most frequently in alternate years (1980–84; Jones et al. 1985).

Meristic and morphological differences in populations of Yellowstone cutthroat trout historically derived from Yellowstone Lake reflect effects of reproductive isolation. Bulkley (1963) examined variation in spotting patterns, basibranchial teeth counts, and coloration as criteria for distinguishing populations. Samples from Yellowstone Lake tributaries (Arnica, Cub, Clear, Chipmunk, Grouse, and Pelican creeks) were compared with those collected from populations that had been separated from Yellowstone Lake for 13 yr (Creston National Fish Hatchery, Montana), 21 yr (Bear Creek), and approximately 8000 yr (Sedge Creek). Yellowstone cutthroat trout in Bear and Sedge creeks were isolated from Yellowstone Lake (and each other) by chemical and thermal barriers when the level of the lake dropped in the early Holocene (Kaplinski 1991); however, Bear Creek was stocked with 252 000 Yellowstone cutthroat trout from the Yellowstone Lake hatchery between 1921 and 1941 (Varley 1981). Based on basibranchial teeth and spotting counts, Sedge Creek and Bear Creek fish differed from one another and from the combined Yellowstone Lake sample and the hatchery sample, and there was evidence of divergence of the Creston hatchery sample from the Yellowstone Lake group. Because differences among populations in spotting pattern and basibranchial teeth number varied directly with time since isolation from Yellowstone Lake, Bulkley (1963) concluded that some degree of genetic change was involved.

In a study of serum-antigen variations among Yellowstone cutthroat trout from seven tributaries and the outlet of Yellowstone Lake, Liebelt (1969) identified at least five separate populations. Fish spawning in Clear, Columbine, and Cub creeks and the outlet were grouped together, but trout from Chipmunk, Grouse, and Pelican creeks and the Yellowstone River inlet all formed unique populations. The isolation of Pelican Creek was attributed to differences in timing of the annual spawning migration, and geographical distance was suggested as a significant factor in the establishment of the other three isolated populations (Liebelt 1969).

Pattern of Spawning Migration

Yellowstone cutthroat trout spawn exclusively in fluvial environments (Varley and Gresswell 1988). Varley and Gresswell (1988) described four migratory spawning patterns for Yellowstone cutthroat trout: fluvial (fluvial residents dispersing locally within the area of the home range); fluvial–adfluvial (fluvial residents moving into tributaries to spawn); lacustrine–adfluvial (lacustrine residents moving into lake tributaries to spawn); and allacustrine (lacustrine residents moving into the outlet stream. The occurrence of all four patterns in the Yellowstone Lake drainage suggests local adaptation to diverse habitats.

The lacustrine–adfluvial migration pattern is most common in the lake drainage, but fluvial and allacustrine patterns have also been observed. For example, fluvial cutthroat trout from the Yellowstone River downstream from the Yellowstone Lake and allacustrine individuals from the lake spawn in the area of the Yellowstone River immediately below the Yellowstone Lake outlet. Tagging studies conducted from 1951 to 1953 suggested little interchange between these populations (Ball and Cope 1961), and recent tagging and marking studies support this conclusion (Kelly 1993). Mechanisms that maintain the putative separation of these two spawning populations have not been extensively investigated, but Ball and Cope (1961) reported temporal separation of the two groups in the spawning area. Fluvial spawning has also been observed in the upper reaches of Pelican and Clear creeks; fish in these areas are assumed to be fluvial residents that remain in these areas throughout their lives.

Yellowstone cutthroat trout spawn between April and August in tributaries of Yellowstone Lake (Ball 1955; Cope 1956, 1957; Jones et al. 1986). Although most individuals in a spawning population are initial spawners, iteroparity is common (Gresswell and Varley 1988). Larger adults generally enter the streams earlier and ascend farther up tributaries than smaller individuals (Cope 1957; Ball and Cope 1961). Ball and Cope (1961) reported that most spawners remain in the tributaries from 1 to 3 wk, and males stay longer than females. This pattern does vary, however, and in 1980, marking studies in Pelican Creek, the second-largest tributary of Yellowstone Lake, suggested that some spawners overwintered in the stream before returning to the lake.

Juvenile Emigration and Residence

Eggs hatch 25–49 d after deposition (278–365 Celsius temperature units, sum of mean daily temperatures above 0°C; Kelly 1993), and young fish emerge from the gravel approximately 2 wk later (Ball and Cope 1961; Mills 1966; Kelly 1993). Most emigrate to the lake within 2 mo of emergence, but some may remain in the natal stream for several years (Welsh 1952; Benson 1960). Immature Yellowstone cutthroat trout that overwinter in Clear, Cub, and Arnica creeks generally emigrate at age 1 or age 2 (Welsh 1952; Benson 1960; U.S. Fish and Wildlife Service, Yellowstone National Park, WY 82190, unpublished data). In Pelican Creek, however, the pattern is more complex. In 1980, all spawners

TABLE 1. Life-history traits of Yellowstone cutthroat trout spawners in six tributaries to Yellowstone Lake, 1950–61.

Stream	Mean spawner abundance[b]	Timing of spawning migration[a]		Mean male:female ratio[c]	Mean age (years)[d]	Mean length (mm)[e]
		Mean initiation (Julian date)	Mean duration (days)			
Pelican Creek	13 282	144	38	0.70:1	4.3	365
Cub Creek	3 793			0.77:1		
Clear Creek	8 938			0.70:1	4.4	378
Chipmunk Creek	8 378	147	56	0.72:1	4.7	391
Grouse Creek	7 362	153	53	0.70:1	4.6	385
Arnica Creek	3 818	131	80	0.65:1	4.3	363

[a]1951–53 (Ball and Cope 1961).
[b]1950–54, 1959, 1961 (Benson and Bulkley 1963).
[c]1950, 1952–53 (Ball and Cope 1961).
[d]1953–55 (Benson and Bulkley 1963).
[e]1953–55, 1958–59 (Bulkley 1961).

migrating upstream (22 293) were marked with fin clips, but 51% of 32 777 downstream migrants (excluding young-of-the-year) were unclipped. Although there are several possible explanations for the large proportion of unclipped fish, multiyear residency is suggested for at least some of these fish because approximately 49% of 2 503 unclipped fish, ranging in total length from 110 to 480 mm, had not spawned.

Raleigh and Chapman (1971) investigated stream temperature, water source, and "innate factors" in relation to migration of fry (young-of-the-year) from incubation areas into Yellowstone Lake. They concluded that movements of fry upstream from the lake outlet (assumed progeny of allacustrine spawners) and downstream from lake tributaries (assumed progeny of lacustrine–adfluvial spawners) were innately controlled responses to environmental stimuli. In a similar study, Bowler (1975) concluded that genetic factors controlled movement of inlet and outlet spawners, but rheotactic acclimation could significantly alter the response of inlet fry. Fry from the outlet area displayed an altered response (downstream movement) when tested in a water source different from the one used for incubation and acclimatization. Although fluvial spawners from the Yellowstone River and allacustrine spawners from Yellowstone Lake both spawn in the outlet area, all fish collected in these studies were presumed to be from allacustrine sources (Raleigh and Chapman 1971; Bowler 1975).

Initiation and Duration of Spawning

Distinct temporal patterns for the spawning migrations in Arnica, Chipmunk, Clear, Grouse, and Pelican creeks during the 1940s and 1950s were identified by Cope (1957). Ball and Cope (1961) reported mean date of spawning initiation and duration of the spawning migration in four of these streams from 1951 through 1953 (Table 1). Statistical analysis (ANOVA, Newman–Keuls multiple-means comparison) of these data reveals significant differences ($p \leq 0.05$) in the mean date of spawning initiation between Arnica Creek (May 10) and Grouse Creek (June 1). Furthermore, mean duration of the spawning migration in Arnica Creek (80 d) differed significantly ($p \leq 0.05$) from Pelican, Chipmunk, and Grouse creeks (38, 53, and 56 d, respectively).

Abundance, Size, and Age of Spawners

Abundance, mean length, and mean age estimates for cutthroat trout spawning in six tributaries of Yellowstone Lake were collected sporadically between 1950 and 1961 (Table 1). The mean number of fish (13 282) ascending Pelican Creek for seven sample years (1950–54, 1959, 1961) was significantly greater (ANOVA, Newman–Keuls multiple-means comparison; $p \leq 0.05$) than all other streams in the subset. Cub and Arnica creeks had the lowest mean estimates (3793 and 3818 fish, respectively).

Differences in mean length of Yellowstone cutthroat spawners sampled in five tributaries were statistically significant (ANOVA, Newman–Keuls multiple mean comparison; $p \leq 0.05$) . Spawners from Chipmunk Creek (391 mm) and Grouse Creek (385 mm) were largest, and those sampled in Arnica and Pelican creeks (363 and 365 mm, respectively) were the smallest. Mean age of spawners for the period between 1953 and 1955 varied from 4.3 yr in Arnica Creek to 4.7 yr at Chipmunk Creek (Table 1); however, differences among streams were not statistically significant (ANOVA, $p = 0.15$).

Male:Female Ratio and Fecundity

The observed male:female ratio for Yellowstone cutthroat trout in a combined sample from 1950, 1952, and 1953 varied from 0.65:1 (Arnica Creek) to 0.77:1 (Cub Creek); observed ratios were identical (0.70:1) for cutthroat trout from Clear, Grouse, and Pelican creeks during this period (Table 1). Differences among streams were not statistically significant (ANOVA, $p = 0.95$).

Cope (1957) used estimates of relative fecundity (number of eggs per unit weight; Bagenal 1978) to compare the size of cutthroat trout eggs among streams. Samples were collected at the Yellowstone Hatchery from 1941 through 1952 (Cope 1957). Differences in slope for regressions of the number of eggs (dependent variable) and ovary weight (independent variable) were statistically significant for Chipmunk, Grouse, and Pelican creeks. Based on these results, Cope (1957) suggested that the relationship between egg size and ovary weight was specific to Yellowstone cutthroat trout from each of the three streams.

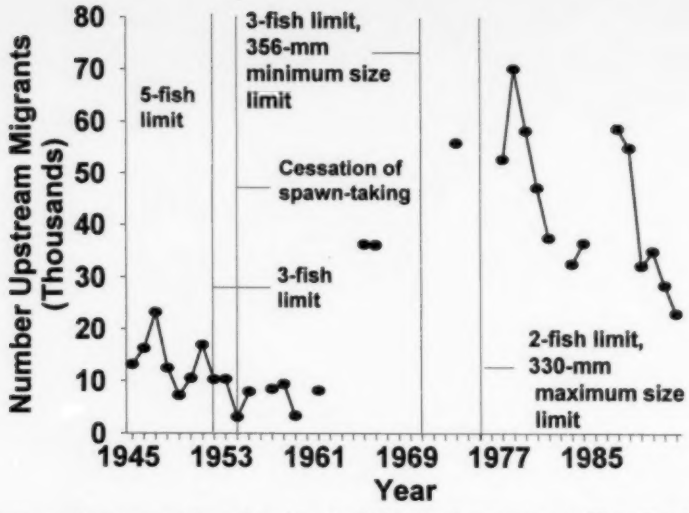

FIG. 3. Counts of Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) spawners at Clear Creek, Yellowstone Lake, under various management scenarios, 1945–92.

Temporal Variation in Life Histories of Cutthroat Trout in Yellowstone Lake

Information describing the spawning population of Yellowstone cutthroat trout in Clear Creek from 1945 to 1992 provides an example of temporal variation in life histories associated with two major anthropogenic activities in the Yellowstone Lake drainage, the hatchery operation and the sport fishery. From 1899 to 1957 over 818 million eggs were collected from Yellowstone cutthroat trout spawners entering tributaries to Yellowstone Lake (Varley 1979). Clear Creek was one of the larger tributaries that were blocked annually for spawn-taking purposes. Mature Yellowstone cutthroat trout congregate in the littoral zone in association with spawning migration and for feeding. Because the majority of angler use is concentrated in the littoral zone, this life-history trait may increase the vulnerability of mature fish to angling (Gresswell and Varley 1988). Immature individuals (through age 2) are not common in the angler catch.

At Clear Creek the number of spawning cutthroat trout declined from a mean of approximately 16 000 between 1945 and 1948 to 3353 spawners in 1954 (Benson and Bulkley 1963). Abundance in Clear Creek increased following implementation of a three-fish (any size) creel limit and the closure of the hatchery and subsequent cessation of spawn taking (Fig. 3). Counts between 1957 and 1961 averaged 7300 spawners annually (Gresswell and Varley 1988), but by the mid-1960s, the number of spawners averaged approximately 36 000 fish annually. After the cessation of spawn-taking activities, the number of Yellowstone cutthroat trout rose despite an increase in the mean number of fish harvested from Yellowstone Lake from 224 000 (1950–53) to 318 000 annually (1954–65).

Following a series of regulations that reduced angler harvest, the number of spawning Yellowstone cutthroat trout continued to increase. A 356-mm minimum-size limit was implemented in 1970. The daily creel limit was three fish until 1973 when it was reduced to two fish. In 1975, a two-fish, 330-mm maximum-size limit was imposed. After the adoption of this regulation, annual harvest dropped to a mean of approximately 71 000 Yellowstone cutthroat trout for the period between 1983 and 1992. Since 1977, annual counts of spawning cutthroat trout at Clear Creek have remained above levels observed in the 1950s (Fig. 3); however, recent declines (1990–92) suggest the influence of factors other than angler harvest on annual variation in spawner abundance.

Mean length of spawning Yellowstone cutthroat trout in Clear Creek averaged approximately 369 mm for the period from 1953 to 1955 (Fig. 4). Annual estimates of mean length increased to an average of 389 mm for the 1957–61 period. As harvest expanded from the late 1950s through the early 1960s, however, mean length of spawners in Clear Creek declined to an average of 365 mm for 1966 and 1967. Since the two-fish, 330-mm maximum-size regulation was instituted in 1975, annual estimates of mean length have risen above previous high measurements and currently average approximately 399 mm (1988–92).

Mean age of Yellowstone cutthroat trout spawning in Clear Creek dropped to a low of 3.9 yr in the mid-1960s concomitant with increasing harvest (Fig. 5). In 1973, only about 6% of the fish sampled were age 6 and over. Since then, mean age has risen substantially, and between 1987 and 1992, mean age for spawners in Clear Creek averaged 5.8 yr. Age-9 Yellowstone cutthroat trout were collected at Clear Creek annually during this period. There is no simple explanation for the observed changes. Increased longevity resulting from decreased angler harvest may have led to an increase in the number of times an individual fish spawned, but delayed maturity could have produced similar results.

Changes in mean length and age have affected the population fecundity of spawners at Clear Creek. Although relative fecundity appears to be unchanged, the current reproductive potential averages almost 32 million eggs annually (Jones et al. 1985). This represents a 500% increase over estimates from the 1950s (6.2 million eggs).

Iteroparity has apparently increased in Clear Creek since the 1950s. Ball and Cope (1961) reported that only 1.22% of

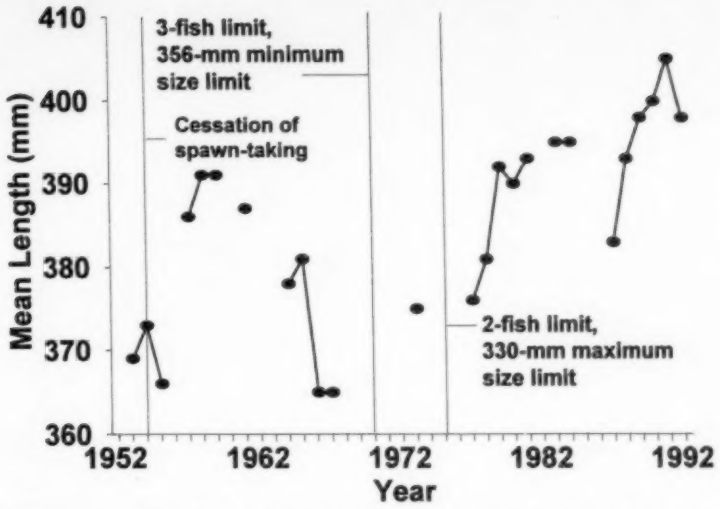

FIG. 4. Mean lengths of Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) spawners entering Clear Creek, Yellowstone Lake, under various management scenarios, 1953–92.

spawning Yellowstone cutthroat trout in Yellowstone Lake were repeat spawners. A mean instream-mortality estimate (five streams over a 5-yr period, 1950–54) of 48.1% indicated that many spawners died after spawning. Returns of marked fish at Clear Creek during the 1980s suggest that a minimum of 23% of fish that spawned in 1979 returned to spawn at least once (Jones et al. 1985), and repeat spawners were collected up to 5 yr after they were originally marked. During five sample years from 1977 through 1984, mean instream-mortality estimated from total upstream and downstream counts in Clear Creek was 12.9%. In recent years, estimates of instream mortality at Clear Creek have increased (mean = 30.8% for 1987, 1988, 1991, and 1992; U.S. Fish and Wildlife Service, Yellowstone National Park, WY 82190, unpublished data); however, the relative influence of changes in monitoring procedures and presence of increased numbers of white pelicans (*Pelecanus erythrorhynchcos*) has not been investigated.

Discussion

Except where barriers limited access, rainbow trout (*Oncorhynchus mykiss*) replaced Yellowstone cutthroat trout in the Columbia River Basin below Shoshone Falls on the Snake River (Behnke 1992) sometime after the falls were formed. Citing Malde (1965), Behnke (1992) suggested that the formation of Shoshone Falls occurred 30 000 – 60 000 yr ago during the Bonneville Flood; however, recent information suggests that this event dates to approximately 14 500 yr ago (Oviatt et al. 1992). At the same time, the Yellowstone subspecies was forced out of high-elevation environments by Pleistocene glaciation. The most recent incursion of Yellowstone cutthroat trout to the Yellowstone River drainage is related to the retreat of glacial ice that occurred about 12 000 yr ago (Richmond and Pierce 1972).

This set of events suggests that the Yellowstone cutthroat trout were reduced to low abundance and greatly restricted range during the late Pleistocene and that current genetic structure of the subspecies was significantly affected by this geographical range constriction. Allozyme data support the occurrence of a geologically recent genetic bottleneck in the Yellowstone subspecies. In a survey of 10 Yellowstone cutthroat trout populations over a broad geographical range, Loudenslager and Gall (1980) reported that only 8% of the genetic diversity was due to divergence among populations. In a broader context, Allendorf and Leary (1988) compared divergence within eight nonanadromous salmonids, and the Yellowstone cutthroat trout was the only form with low genetic divergence among local populations.

There are inconsistencies in the taxonomy of cutthroat trout, however. Although the Yellowstone and west-slope (*Oncorhynchus clarki lewisi*) subspecies have been isolated from each other for 1–2 million yr (Allendorf and Leary 1988; Behnke 1988) and allozyme data suggest that west-slope cutthroat trout may be a distinct species (Allendorf and Leary 1988), morphologically the two subspecies remain undifferentiated (Allendorf and Leary 1988). Alternatively, life-history traits of spawning populations of Yellowstone cutthroat trout within the Yellowstone Lake drainage suggest phenotypic variation that may imply local adaptation, but allozyme divergence is low compared with that observed among populations of westslope cutthroat trout (D.K. Shiozawa and R.N. Williams, Department of Zoology, Brigham Young University, Provo, UT, unpublished data).

One exception to this generality was detected in genetic divergence of Yellowstone cutthroat trout from Sedge Creek (R.N. Williams, R.F. Leary, and D.K. Shiozawa, Clear Creek Genetics, Meridian, ID, unpublished data). This population has been reproductively isolated for thousands of years, and heterozygosity and allelic diversity were substantially lower within the Sedge Creek population than in other populations of Yellowstone cutthroat trout. Samples of cutthroat trout from Sedge Creek and Yellowstone Lake revealed substantial allozyme divergence (Nei's $D = 0.046$) compared with levels commonly observed among other Yellowstone cutthroat trout populations (Nei's $D = 0.003$); however, no evidence of divergence of mitochondrial DNA between Sedge Creek and Yellowstone Lake populations was apparent.

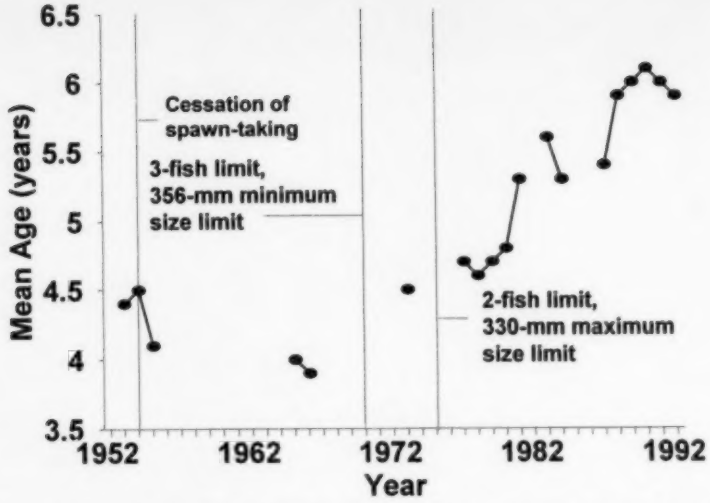

FIG. 5. Mean age of Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) spawners entering Clear Creek, Yellowstone Lake, under various management scenarios, 1953–92.

These examples underscore difficulties that arise in the management and conservation of polytypic taxa such as the cutthroat trout. Lack of genetic divergence (based on solely on molecular analysis) could suggest that local adaptations are simply phenotypic responses to environmental variability and not the basis for conservation of local populations. Allendorf and Leary (1988) concluded, however, that lack of divergence at allozyme loci is not indicative of the absence of important interpopulation genetic differences. Considering efforts to preserve genetic diversity, Echelle (1991) cautioned that no single measure of variation should be considered exclusively. Identifying life-history differences between populations can provide important information concerning local adaptation and the relationship among life-history organization and specific aspects of the environment. Documentation of life-history differentiation is necessary to provide management support for the protection of unique life-history types in the absence of documented allozyme divergence.

The presence and extent of the life-history diversity in Yellowstone Lake may be important, regardless of the mechanisms that control it. Numerous studies suggest that life-history variation has adaptive significance in other polytypic fish species (e.g., Healey 1986; Taylor 1991; Hankin et al. 1993; Quinn and Unwin 1993), and phenotypic plasticity can be a key component of evolutionary change (Thompson 1991). Although genetic differentiation is apparently low among Yellowstone cutthroat trout, the documented life-history variation suggests a strong adaptability to disparate environments. Den Boer (1968) submitted that diversity is directly related to the probability of long-term population persistence; in a spatially and temporally heterogeneous environment, spreading risk through maintenance of natural diversity is critical. Diversity provides a means of buffering environmental change at all levels of differentiation (i.e., within a species, subspecies, metapopulation, and population), and therefore, management activities that impinge on the natural variation in a polytypic taxon may eventually reduce the capacity for survival of the taxon as a whole.

From an evolutionary standpoint, local adaption provides an important means to exploit a wide variety of habitats. A metapopulation comprises numerous locally adapted populations. At any point in time, some populations may be declining in abundance while others are becoming established or increasing (Hanski and Gilpin 1991). These opposing processes of extinction and colonization determine the trajectory of metapopulation persistence. For a metapopulation to persist, the rate of population extinctions must not exceed the rate of colonization. From this perspective, local populations within a metapopulation have important functional value in metapopulation persistence regardless of their genetic or phenotypic uniqueness.

The relative integrity of the cutthroat trout metapopulation structure in Yellowstone Lake may have influenced recovery of the fishery when constraints imposed by angler harvest diminished. Management policies of the U.S. National Park Service contributed to the pristine condition of the Yellowstone Lake watershed and spawning and rearing habitats and, consequently, to the integrity of the cutthroat trout metapopulation. The uneven effects of angling also supported the structure of the metapopulation. Because angler effort and harvest were not spread evenly throughout the lake (Benson and Bulkley 1963), it is likely that some of the spawning populations were relatively unaffected by harvest. Additionally, some life-history types (e.g., those with early spawning migration or extended stream residence) within heavily impacted populations may have persisted.

A wide range of life-history diversity occurs among cutthroat trout populations spawning in tributaries of Yellowstone Lake. These differences are spatially distributed among the spawning populations, and temporal variation within the Clear Creek spawning populations coincided with changes in management strategies. Life-history variation in the Yellowstone Lake drainage is reflective of diversity that has been documented at the subspecies and species level (Gresswell 1988).

Of the life-history characteristics examined, abundance and mean length of spawners and timing (i.e., initiation, duration, and peak) of annual spawning migrations appear to

be the most variable in the Yellowstone Lake drainage. Male:female ratio was similar among streams, and although some annual variation has been noted, no temporal trends have been identified. Differences in mean age among spawners from various tributaries were not significant when data collected from six streams during the 1950s was compared; however, these data may have been affected by changes in population structure resulting from anthropogenic activities during that period. For example, mean age of spawners at Clear Creek increased almost 2 yr after cessation of spawn taking and reductions in harvest during the 1970s.

Life-history organization of spawning populations of cutthroat trout in Yellowstone Lake is complex, but evidence suggests a relationship with spatial and temporal aspects of the environment. Preliminary investigation of basin-level environmental variables and life-history parameters obtained from 27 tributaries between 1985 and 1987 suggests that elevation, aspect, and drainage area are correlated with the date of spawning migration initiation and mean length of cutthroat trout spawners in individual tributaries (R. Gresswell, unpublished data). Elevation, aspect, and drainage area are among physical characteristics of drainages that directly influence flow and peak discharge (Morisawa 1968), and the timing of spawning migrations appears to be directly related to hydrographic stage and water temperature (e.g., Ball and Cope 1961; Jones et al. 1990). Relationships between spawning migrations and hydrographic pattern thus suggest one possible link between habitat variation and life-history diversity in the Yellowstone Lake drainage.

Habitat complexity of Pelican Creek may explain migration patterns such as extended residence in the stream 3 or 4 yr after emergence or spawning adults that remain in the stream for months or years before emigration. Pelican Creek has a mainstem length of over 53 km and at least 125 first-order tributaries. Hydrothermal springs that are locally numerous in some of the tributaries to Pelican Creek may provide refuges for overwintering fish. Additionally, resident populations of fluvial Yellowstone cutthroat trout have been observed in Pelican Creek. Apparently, many of the life-history types occurring throughout the Yellowstone Lake drainage are present in Pelican Creek alone.

Temporal variation of life-history traits at Clear Creek is strongly associated with changes in fishery management. Because increases in the number of spawners at Clear Creek after the cessation of spawn-taking activities occurred while angler harvest was increasing, it is probable that spawn-taking operations contributed to previous reductions in spawner abundance. Alteration of the historical gene pool or reduction in the number of cutthroat trout allowed to proceed upstream to spawn naturally may have contributed to this decline. Similar effects from hatchery introductions have been documented for a wide range of salmonids in North America (Evans and Willox 1991; Krueger and May 1991), and the negative effects of hatchery supplementation is a principal consideration in the conservation of dwindling wild populations (Ryman and Laikre 1991; Waples 1991)

Declines in mean length and age of spawning Yellowstone cutthroat trout at Clear Creek during the 1960s may be associated with fish harvest by anglers in Yellowstone Lake. Alterations observed in the structure of the Clear Creek population are probably representative of the magnitude of change that has occurred in other lacustrine–adfluvial spawning populations in Yellowstone Lake, and data collected from spawning cutthroat trout at Pelican and Cub creeks during the late 1970s and early 1980s suggest similar responses to reduction in harvest (Jones et al. 1979, 1983). Patterns from the Yellowstone Lake fishery are consistent with effects of harvest predicted by population theory (Nikolskii 1969) and with observations from commercial fisheries (Christie 1972; Larkin 1977; Ricker 1981). Resulting reductions of mature fish in a population can drastically reduce the reproductive capacity, especially within late-maturing and iteroparous life-history types (Ricker 1963). Less abundant forms are at increased risk of extirpation or extinction (Nelson and Soulé 1987).

There is little evidence that the introduction of non-native fishes has negatively affected Yellowstone cutthroat trout in Yellowstone Lake (Gresswell and Varley 1988). Some diet overlap has been identified, but spatial interaction is low among these fishes. It is fortuitous that non-native salmonids have not become established in the drainage because similarities in habitat requirements and life-history traits common to this family of fishes often result in intense interspecific competition (e.g., Marrin and Erman 1982; Griffith 1988). Additionally, hybridization with rainbow trout could potentially result in the extirpation of the indigenous Yellowstone cutthroat trout genome in Yellowstone Lake.

Attempts to accommodate continued increases in visitation to Yellowstone National Park may eventually threaten the integrity of the Yellowstone Lake ecosystem and ultimately, the cutthroat trout metapopulation in the lake. Development of visitor facilities at Lake Village, Bridge Bay, Fishing Bridge, and Grant Village has included a substantial road system. Road culverts have reduced access to adfluvial spawners in some tributaries, and at least two culverts totally block annual spawning migrations (Dean and Varley 1974; Jones et al. 1986). Disturbance of spawners, trampling of eggs in redds (Roberts and White 1992), and angling mortality (Gresswell and Varley 1988) are perturbations associated with the presence of visitors in the vicinity of spawning streams. Additionally, human presence may alter use patterns of natural predators, affecting both predator and prey populations (Swenson 1975; Reinhart 1990). This is especially important because of the frequent occurrence of endangered species including grizzly bears (*Ursus arctos horribilis*) and bald eagles (*Haliaeetus leucocephalus*) in the Yellowstone Lake drainage. Alteration of natural fire regimes, especially in the developed areas, has also occurred in the drainage. Although individual activities such as these may not appear to have substantial effects on the persistence of the cutthroat trout in the Yellowstone Lake drainage, the synergistic and cumulative effects of human presence should be considered in all future management decisions. Each individual spawning population is important in the spatiotemporal mosaic of populations and life-history types that form the Yellowstone Lake metapopulation and ultimately to the persistence of this complex assemblage.

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Genetic Risk Associated with Supplementation of Pacific Salmonids: Captive Broodstock Programs

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Waples, R.S., and C. Do. 1994. Genetic risk associated with supplementation of Pacific salmonids: Captive broodstock programs. Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 310–329.

We simulated some of the genetic consequences of temporarily using captive broodstocks to supplement Pacific salmon (*Oncorhynchus* spp.) populations. Results were summarized in terms of the parameter ΔIBD, which represents the change in level of inbreeding in the postsupplementation population compared with the control. We found that: (1) the most important factor affecting ΔIBD was whether the population remained large after supplementation ceased; (2) the absolute number of wild adults taken for broodstock had a stronger influence on ΔIBD than did the proportion of the population sampled; (3) if a captive broodstock program is successful, virtually all of the genes in the postsupplementation population will be derived from fish taken into the hatchery for broodstock; (4) in programs that last longer than one generation, marking hatchery fish is essential to avoid additional increases in inbreeding, and marking rates <100% may be ineffective; and (5) broodstock practices such as sib-avoidance mating and equalizing progeny number affected inbreeding levels during the captive phase but had little permanent effect on ΔIBD. A number of factors not considered in this study (e.g., domestication selection and the fitness consequences of given levels of inbreeding) should also be evaluated in deciding whether or how to implement captive broodstock programs.

Les auteurs ont effectué une simulation des conséquences génétiques de l'utilisation temporaire de géniteurs gardés en captivité pour augmenter les populations de saumon du Pacifique (*Oncorhynchus* spp.). Ils ont résumé les résultats en termes du paramètre DIBD, représentant le changement du niveau d'endogamie dans la population après introduction de géniteurs gardés en captivité par rapport au groupe témoin. Ils ont trouvé que : 1) le plus important facteur ayant une incidence sur le DIBD était l'abondance de la population après la fin de l'apport en géniteurs d'élevage; 2) le nombre absolu d'adultes sauvages capturés pour obtenir des géniteurs d'élevage avait une plus grande incidence sur le DIBD que le pourcentage de la population échantillonnée; 3) si un programme d'élevage de géniteurs réussit, presque tous les gènes dans la population post-apport de géniteurs d'élevage proviendront des saumonds capturés pour obtenir des géniteurs d'élevage; 4) dans le cas de programmes qui s'étendent au-delà d'une génération, il est essentiel de marquer les géniteurs d'élevage afin d'éviter un nouvel accroissement de l'endogamie, et il se peut qu'un niveau de marquage <100% ne soit pas efficace; 5) bien que les techniques d'élevage de géniteurs comme la prévention des croisements frères-soeurs et la régularisation de la progéniture ont eu une incidence sur le niveau d'endogamie pendant la phase de captivité, elles ont eu peu d'incidence permanente sur le DIBD. D'après les auteurs, il faudrait évaluer un certain nombre d'autres facteurs qu'ils n'ont pas examinés (p. ex. le choix des saumons d'élevage et les conséquences de niveaux donnés d'endogamie sur la valeur adaptative) avant de décider de la mise en oeuvre d'un programme d'élevage de géniteurs et de ses modalités.

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The use of artificial propagation with Pacific salmon, *Oncorhynchus* spp. has a long history (Lichatowich and McIntyre 1987). Efforts have primarily focused on sea ranching—that is, incubating eggs in hatcheries and rearing juveniles until they are released, prior to or at the onset of smolt development. Because egg-to-smolt mortality is typically high in the wild, successful salmon hatcheries provide a considerable survival advantage in the early life stages that may translate into substantial increases in the number of returning adults. For much of this century, hatcheries have been used to mitigate declines or extirpation of stocks due to habitat degradation, overfishing, and blockage of migratory routes. More recently, there has been increasing interest in the use of hatchery-reared fish to help restore the abundance of natural populations. This approach is known as supplementation and generally involves collection of some fraction of the wild population for hatchery broodstock, with progeny of hatchery matings released into the wild to supplement the population.

Recent concern regarding the decline of natural populations of Pacific salmon has spurred interest in another approach to artificial propagation: rearing individuals to maturity in captivity, as in a trout hatchery. This approach, known as a captive broodstock program, is practiced commercially in large net-pen operations in the Pacific Northwest and elsewhere. The use of captive broodstocks for supplementing natural populations, however, is still largely experimental. Nevertheless, such programs are already being used in recovery

efforts for Sacramento River winter-run chinook salmon (*O. tshawytscha*) and Snake River sockeye salmon (*O. nerka*), which are listed as endangered under the U.S. Endangered Species Act.

Captive broodstock rearing is an attractive concept because it has the potential to rebuild depleted natural populations rapidly. Whereas traditional salmon hatcheries provide a survival advantage only in the egg-to-smolt stage, captive broodstocks may greatly improve smolt-to-adult survival as well. For example, assuming survival rates in captivity of 80% egg-to-smolt and 25% smolt-to-adult, a single female with fecundity of 5000 eggs could produce 1000 captively reared adults in one generation. Thus, not only is it theoretically possible to expand the population size very quickly, it might not be necessary to take many individuals from the wild to do so.

A number of issues, however, remain to be resolved before captive broodstocks can be endorsed as a general method for restoring salmon populations. First, as yet there is little empirical evidence to show that supplementation (whether using sea ranching or captive broodstocks) can produce stable increases in the abundance of natural salmon populations. Second, several technical and logistical problems related to rearing Pacific salmon to maturity in captivity remain to be resolved. These include methods to ensure adequate health and survival, maturation issues such as gamete formation and viability, and the choice of fresh or salt water for rearing. Third, selective regimes in captivity differ from those in the wild, and the degree to which this will affect viability of the postsupplementation population is not well understood.

Recently, Ryman and Laikre (1991) pointed out another possible deleterious genetic effect of supplementation, which they referred to as supportive breeding: a reduction in effective population size that may result from preferentially augmenting that part of the gene pool taken into the hatchery. The concern is that if the supplementation program is successful, a large fraction of the individuals in the postsupplementation population will be derived from a relatively small number of "founders" in the initial population. The result, in effect, can be an artificial genetic bottleneck that can lead to increased levels of inbreeding and erosion of genetic diversity. We refer to this phenomenon as the Ryman–Laikre effect. Because of their potential to rapidly magnify a small portion of the population, captive broodstocks are particularly prone to the risks posed by the Ryman–Laikre effect.

Ryman and Laikre made a significant contribution in raising awareness of this issue and in illustrating how, in supplementation programs, the effective size of the hatchery–wild population as a whole is generally more important than the size of either component alone. Nevertheless, their analysis was limited in that it considered only a single generation of supplementation and did not consider age structure. Neither did they consider a situation comparable to a captive broodstock program, in which two (or possibly more) rounds of breeding occur in captivity. Finally, Ryman and Laikre did not distinguish between scenarios in which the population remains large after supplementation and those in which the increase in population size is not sustained (Fig. 1). Although the latter scenario clearly can lead to a genetic bottleneck and reduction in effective population size, it is not so clear what the genetic consequences would be if the population size remains large. If supplementation simply added fish to the population without depressing production of fish left to spawn naturally, then there would be no loss of genetic variation, although allelic and genotypic frequencies might have been altered.

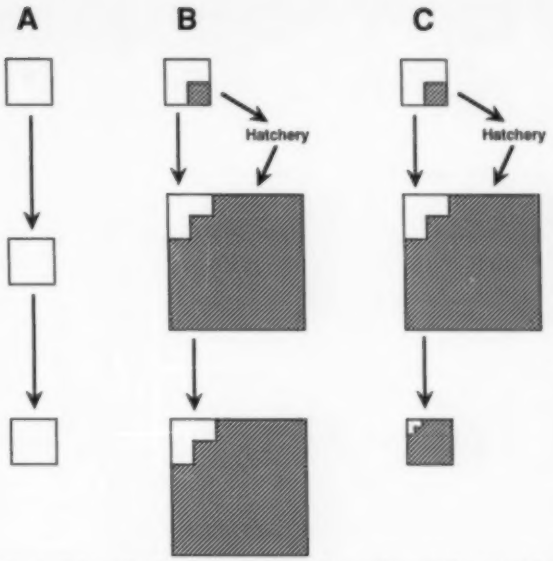

FIG. 1. Schematic diagram of three basic supplementation scenarios considered in this paper. (A) No supplementation (control). (B) Captive broodstock program used to increase survival of progeny of fish taken into hatchery. Productivity of fish remaining in wild is unchanged (increase scenario). (C) As in B, but either "surplus" fish are harvested or numbers exceed carrying capacity, so that abundance drops to presupplementation levels after program is terminated (crash scenario).

To examine in more detail the genetic consequences of using captive broodstocks to supplement Pacific salmon populations, we used computer simulations based on a model that incorporates life-history features common to most species of Pacific salmon (notably, semelparity with overlapping year classes). We monitored genetic parameters in the modelled population as they changed over time in response to factors such as duration of the captive broodstock program, strategies for broodstock collection, strategies for mating captively reared fish, marking rate of hatchery fish, and carrying capacity of the environment. Results showed that the genetic consequences of captive broodstock programs are complex and can be sensitive to the factors identified above.

Although the present study was intended to provide a semicomprehensive analysis of the phenomenon described by Ryman and Laikre (1991), it was by no means a comprehensive genetic analysis of captive broodstock programs. The present analysis focussed entirely on random processes that affect selectively neutral alleles. These random processes are important because they can strongly influence the rate of inbreeding and the rate of loss of genetic variability. Nevertheless, several important factors were not considered here. In particular, results presented here tell us nothing directly about the short- or long-term fitness consequences of increased levels of inbreeding. Furthermore, we have not attempted to evaluate the effects of genetic changes due to altered selective regimes experienced by captively reared fish. These are complex issues that deserve full attention elsewhere.

TABLE 1. The standard parameter set used in the simulations. In general, alternative scenarios were evaluated by changing a single parameter from the list below.

Parameter	Abbreviation			Standard value
Maximum age at reproduction	M			5
Number of wild spawners per year in initial population	N			50
Year hatchery starts	—			25
Year hatchery ends	—			29
Number of adults per year taken into hatchery	NH			10
Number of progeny per captured female that survive to reproduce in captivity (fixed)	CP			1000
Mean number of progeny per captive female that return as adults following release from hatchery (random variation)	HP			2
Proportion of hatchery fish marked	—			1.0
Initial allele frequency	P			0.5
Proportion maturing at age:				
		3	4	5
Progeny of naturally spawning fish		0.25	0.50	0.25
Captively reared fish		0.75	0.25	0
Hatchery fish released as juveniles		0.60	0.30	0.10
Additional aspects of the standard model				
Sib-avoidance mating practiced in hatchery				
Sex ratio random for fish captured from wild				
Natural production limited to equilibrium population size of $K = N$ fish per year regardless of number of returning adults				
A single, closed population; no mutation, migration, or selection				
Other notation used in this paper				
Effective population size per generation	N_e			
Effective number of breeders per year	N_b			
Inbreeding and variance effective population size	N_{ei}, N_{ev}			
Inbreeding and variance effective number of breeders	N_{bi}, N_{bv}			
Effective size of captive and wild phases	N_{ec}, N_{ew}			
Effective number of breeders in captive and wild phases	N_{bc}, N_{bw}			
Equilibrium population size with density dependence	K			
Population growth rate with no density dependence	α			
Density-dependence scaling factor	β			
Mean number of progeny per parent that survive to reproduce	$\bar{k}$			
Variance in number of progeny per parent that survive to reproduce	V_k			
Probability that two alleles in an individual are identical by descent	IBD			
Change in IBD in postsupplementation population attributable to the captive broodstock program	ΔIBD			

A companion study (B.E. Riddell and A. Cass, Department of Fisheries and Oceans, Nanaimo, B.C., and R.S. Waples, National Marine Fisheries Service, Seattle, Wa, unpublished data) addresses the Ryman–Laikre effect as it applies to traditional sea ranching programs for supplementing wild populations of Pacific salmon.

The Model

The computer simulations were carried out using a PASCAL program that was similar in some respects to models previously used to evaluate genetic changes over time in salmon populations (Waples and Teel 1990; Waples 1990a, 1990b). The variables and notation used in the model and in the results are shown in Table 1. The simulations were initialized by choosing N breeding fish in each of M years, with M being the maximum age at reproduction. Genotypes for fish in the initial set of M years were determined by randomly drawing a pair of gametes from an initial gamete pool in which the frequency of the A allele was an input parameter (P_A). In addition, each of the $2NM$ total alleles in the initial population was assigned a unique number (e.g., fish 1 in year 1 had alleles 1 and 2, fish N in year 1 had alleles $2N - 1$ and $2N$, and fish N in year M had alleles $2NM - 1$ and $2NM$). This allowed us to monitor the rate of increase in identity by descent (IBD), which is the probability that the two alleles in an individual were descended from the same copy of an allele in a previous generation (Crow and Kimura 1970). This probability is known as the inbreeding coefficient of a population. In our simulations, the incidence of IBD could be measured by counting the proportion of individuals containing two alleles having the same number. Homozygosity, in contrast, depends on whether the two alleles in an individual are of the same type (in this

case, A or B), regardless whether they originated from the same ancestor.

Simulations were run for a total of 75 yr. During the initial period (years $M + 1$ to 25), the natural population was allowed to reach a dynamic equilibrium in the rate of genetic change per year. Matings in the wild were simulated using a Wright–Fisher model with separate sexes. The sex of each individual was assigned at random, and each mating produced one offspring and involved one randomly chosen male and one randomly chosen female, with replacement. Under this model, within each sex the variance among individuals in reproductive success is binomial. If sex ratio were fixed at 1:1, the spawners each year would be an "ideal" population in which the effective number of breeders (N_b) is equal to the total population (N). Because sex ratio was allowed to vary randomly, N_b was less than N. As demonstrated in the Appendix, the expected value for N_b under this model is $N - 1$.

Matings in the wild each year produced progeny according to recruitment functions described below. In the "standard" model, the total number of progeny produced that survived to adulthood was the same as the number of spawning adults. Thus, N progeny were produced in each of the initial M years, but the number of spawning adults in the years after year M varied somewhat because the age at reproduction for each progeny was chosen randomly, based on a vector of age-structure parameters read as input variables (see below). To ensure that all alleles in the first M years were unique, progeny produced in the first few years that returned to spawn prior to year $M + 1$ were ignored.

Beginning in year 25 and continuing for a variable number of years, a number of adult fish (NH) were taken from the returning natural fish for use in the captive broodstock program. Fish to be taken into the hatchery were chosen randomly with respect to age and sex, except for the stipulation that at least one of each sex was taken. Fish not taken to the hatchery were allowed to spawn naturally and produce a number of progeny determined by the recruitment function.

To minimize variance in reproductive success among adults taken into the hatchery, each female produced the same number (CP) of progeny that survived to reproduce in captivity. If the sex ratio was equal, pairwise matings were used, with the pairings chosen randomly; if sex ratio was not equal, at least some members of the less numerous sex participated in multiple matings, but the difference in number of matings among individuals of the same sex was never more than one.

For individuals that matured in captivity, we used a modified random-mating design with some proscribed matings. This design was intended to avoid full- and half-sib matings while keeping the mean number of matings per individual of each sex from departing too much from the sex ratio. Satisfying both criteria simultaneously (pseudorandom mating with no sib matings) was surprisingly difficult; in general however, we accomplished pseudorandom mating with little or no sib mating. Sex-ratio differences among captively reared adults were handled as described above for natural adults brought into the hatchery as broodstock.

Captively reared adult females produced an average of HP hatchery progeny that were released as juveniles and returned as adults. Because there is essentially no opportunity to control family size after release from a hatchery, the number of surviving progeny per female spawner was chosen as a random variable with binomial variance. Progeny released from the captive broodstock program that survived to adulthood were allowed to mate randomly with the natural population. In some simulations, progeny of the captive broodstock program returned as adults before broodstock collection had ceased. These progeny were subject to broodstock collection unless they had been marked as hatchery fish, in which case they were allowed to spawn naturally. The proportion of hatchery fish that were marked ranged from 0 to 1.

The age at reproduction for all captive and wild progeny was chosen randomly, based on vectors of the proportion of the population maturing at each age. Separate vectors were used for progeny of natural spawnings, progeny reared to maturity in captivity, and progeny released from the hatchery as juveniles.

When progeny released from the hatchery began to return as adults, the size of the natural population rapidly increased. Subsequently, abundance of the natural population was allowed to follow one of two general paths (Fig. 1). In the increase scenario, the population was allowed to remain large after termination of the captive broodstock program. This might occur if the population simply needed a temporary "jump start" to send it into a higher level of production. An implicit assumption in the increase scenario was that either carrying capacity was not limiting abundance in the first place, or problems impeding recovery were resolved prior to termination of the captive broodstock program.

In the crash scenario, the population was allowed to decline to the initial level (N) following termination of the captive broodstock program. This might occur if the low initial abundance was primarily a consequence of limited carrying capacity, and if habitat restoration was not addressed in conjunction with the captive broodstock program. We considered both a precipitous decline to the original population size (i.e., even a very large number of naturally spawning adults produced only a total of N progeny that survived to reproduce) as well as the generally more gradual declines governed by Ricker and Beverton–Holt recruitment functions.

Parameters of the Standard Model

One thousand replicates were performed for each parameter set defining a simulation. Because a large number of factors could affect the results of the simulations, it was not possible to consider all possible permutations of different parameter values. To make the analysis feasible without sacrificing comprehensiveness, we selected a "standard" parameter set (Table 1) to compare in sequence with a variety of alternate scenarios. Briefly, the rationale for the standard parameter set is as follows.

The small number of natural spawners per year ($N = 50$) reflects the assumption that, in general, a captive broodstock program will only be considered if population size is small. The small number of wild adults taken to the hatchery each year (NH = 10) reflects widespread concern for minimizing risk to the natural population from broodstock collection, as well as the possibility of producing a large increase in abundance from just a few fish. The standard value for number of progeny per captured female that survived to spawn in captivity (CP = 1000) could be achieved with approximately 20% egg-to-adult survival from a female with a fecundity of 5000 eggs (a typical value for chinook salmon). The mean number of surviving progeny produced by each captively reared female (HP = 2) would correspond to a

smolt program that was merely operating at replacement level. The value of CP was fixed (invariant among females), reflecting the opportunity in a hatchery to limit variation in family size. In contrast, for reasons discussed above, we considered random (binomial) variation around the mean value of HP.

The standard model involved collection of broodstock for only 5 yr (years 25–29), which is the maximum life-span for many populations of Pacific salmon. The age at maturity for natural fish (50% age 4 and 25% each ages 3 and 5) was considered reasonable for many species of Pacific salmon and is the same age structure used in a number of previous simulation studies of genetic change in Pacific salmon populations (Waples 1990a, 1990b; Waples and Teel 1990). Age at maturity of hatchery fish released as juveniles (60% age 3, 30% age 4, and 10% age 5) reflects the shift toward younger age at maturity that has been observed in many salmon hatchery populations (Hager and Noble 1976; Bilton 1984; Ritter et al. 1986). Age at maturity of captively reared fish (75% age 3 and 25% age 4) was obtained by assuming that all fish would mature one year earlier in captivity than they would have in the wild, with the provision that no fish matured prior to age 3.

In the standard model, all hatchery fish released as juveniles were marked so that they could be identified and not taken for broodstock if they returned as adults. Full- and half-sib family groups were kept separate in the captive-rearing phase, and sib-avoidance matings were practiced to the extent possible. The sex ratio of wild fish captured for broodstock was random, as would be the case if fish were collected before they show obvious sexual dimorphism or if there were restrictions on the total number of wild fish that can be captured or handled. Initial allele frequency for the single locus under consideration was 0.5 in the standard model except in those runs designed to analyze the rate of loss of rare alleles.

Genetic and demographic parameters monitored each year in the natural population (before collection of hatchery broodstock) included total number of spawners, allele frequency, observed heterozygosity, identity by descent, allelic extinction, and variance in allele frequency. An allele was declared extinct if its frequency was 0 in M consecutive years. The variance in allele frequency was computed as the sample variance (among replicates) in allele frequency at a given point in time (e.g., after 25, 50, or 75 yr). Under the conditions used in the model, allele frequency was expected to change randomly, heterozygosity was expected to decrease over time, and identity by descent and variance in allele frequency were expected to increase.

Results

Changes in IBD Under the Three Scenarios

As the goal of this study was to examine the effects of captive broodstock programs on overall levels of inbreeding in a population, our primary focus was on changes in the inbreeding coefficient, or IBD. Because each allele in the initial population was unique in our simulations, IBD was initially zero and we could monitor the increase in IBD directly.

It is important to note that IBD can change for two related, but distinct, reasons. First, IBD will increase as a result of genetic drift, which leads to a continual loss of individual alleles through random processes. In a randomly mating

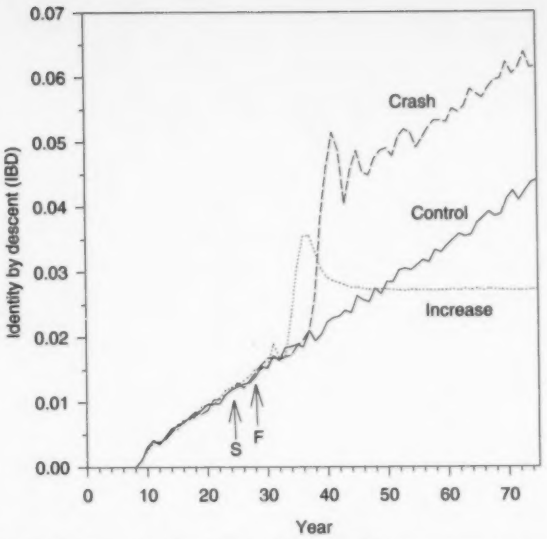

FIG. 2. Increase in identity by descent (IBD) over time in simulations using the three scenarios depicted in Fig. 1. Except for variations applicable to the control, crash, or increase scenarios, input parameters were as shown in Table 1. S and F indicate the start and finish, respectively, of broodstock collection.

population of fixed size, IBD inevitably increases as the number of distinct alleles decreases. This increase in IBD is permanent unless new alleles are generated through mutation or are introduced through immigration, two processes not considered in this model. Second, IBD can either increase or decrease as a result of assortative mating, in which related individuals breed with each other more (or less) often than would occur by chance. Changes in IBD due to this phenomenon are temporary because they merely reflect rearrangement of alleles within the population rather than permanent loss of alleles. A subsequent episode of random mating will erase changes in IBD due solely to assortative mating. In this paper, we consider only changes in IBD due to genetic drift.

According to theory (e.g., Crow and Kimura 1970), the proportional increase in IBD due to random genetic drift is $1/(2N_e)$ per generation, and IBD is expected to increase from zero to $1 - [1 - 1/(2N_e)]^t$ after t generations. Our simulation model could be tested against this theory by examining results for a control run of the standard parameter set with no captive broodstock program (i.e., NH = 0). To calculate the expected value of IBD at the end of the simulation (year 75), we must know N_e and the elapsed number of generations. Because each allele in the initial population was unique, IBD was 0 in years 1–5. Furthermore, because selfing was not allowed in our model, IBD did not begin to increase until the second generation following the initial 5 yr. Generation length (average age at reproduction; g) in the standard model was 4 yr, so our model measured inbreeding that accumulated over $75 - (5 + 4) = 66$ yr, or $t = 16.5$ generations. As noted previously, the effective number of breeders per year (N_b) under the standard model was $N - 1 = 49$. For Pacific salmon populations, Waples (1990a) showed that effective population size per generation (N_e) is equal to gN_b, so N_e was $4 \times 49 = 196$ in the standard model. Substituting $t = 16.5$ and $N_e = 196$ into Crow and Kimura's

TABLE 2. Expected number of adults returning to spawn each year under the standard parameter set with the crash scenario (natural production constant at $N = 50$ fish per year). Age structure of captive and wild fish is shown in Table 1. Hatchery production is shown for groups derived from brood years for initial broodstock collection (years 25–29) and for maturation in captivity (years 28–33).

		Hatchery produced: Initial brood year / Captive brood year						
		25	25+26	26+27	27+28	28+29	29	Total
Year	Naturally produced[a]	28	29	30	31	32	33	natural spawners
1–30	50 (0)	—	—	—	—	—	—	50
31	50 (0)	2250	—	—	—	—	—	2300
32	50 (0)	1125	3000	—	—	—	—	4175
33	50 (0)	375	1500	3000	—	—	—	4925
34	50 (25)	—	500	1500	3000	—	—	5050
35	50 (75)	—	—	500	1500	3000	—	5050
36	50 (99+)	—	—	—	500	1500	750	2800
37	50 (99+)	—	—	—	—	500	375	925
38	50 (99+)	—	—	—	—	—	125	175
39–75	50 (99+)	—	—	—	—	—	—	50
Total		3750	5000	5000	5000	5000	1250	

[a]Parenthetical numbers represent the percentage of naturally produced fish derived from hatchery fish that spawned naturally.

formula indicates that IBD should increase to approximately 0.0413 by year 75 with $N = 50$ and no captive broodstock program. The actual value observed for the control scenario (IBD ≈ 0.043; Fig. 2) was in close agreement with this theoretical value.

Figure 2 also shows the pattern of increase in IBD for the increase and crash scenarios shown in Fig. 1. In the increase scenario, the population remained large ($N \approx 5000$) following supplementation; in the crash scenario, the population fell precipitously to its original size following termination of the program. Results for the two scenarios were quite different. If the population remained large following supplementation (i.e., if carrying capacity was not a limitation, and if "excess" fish were not harvested), the temporary increase in IBD that resulted from the captive broodstock program was quickly offset by reduced levels of inbreeding due to a large population size. As a result, after approximately year 50 the cumulative level of inbreeding was less than would have been expected in the natural population if no captive broodstock program had been initiated (and no other steps taken to increase carrying capacity or productivity).

In contrast, if the population crashed back to its original size ($N = 50$) following termination of the broodstock program, IBD was considerably higher than in the control scenario (approximately 0.063 vs. 0.043 after 75 yr). Note that, in the crash scenario, the increase in IBD occurred over a short time period; after a period of adjustment, the rate of increase was the same as in the control population because after supplementation both scenarios had the same total and effective population sizes.

Some explanation of the detailed form of the curves for the increase and crash scenarios shown in Fig. 2 is important, not only for clarity but also to illustrate the complexity of the problem considered here. Identity by descent and other parameters were monitored in the population of adults returning to spawn in the wild each year. Although broodstock collection in the standard model occurred in years 25–29, the increase in IBD was not seen in the natural spawners until year 34 in the increase scenario and year 38 in the crash scenario.

This difference can be explained as follows. Adults collected in year 25, the first year of the captive broodstock program, produced progeny that matured in captivity at ages 3 (75%) and 4 (25%) (Table 2). Three-year-old captively reared fish maturing in year 28 were all derived from only 10 adults and therefore included many full and half sibs. However, because sib-avoidance mating was practiced in the hatchery, progeny of matings in year 28 were not inbred. Nevertheless, these progeny were closely related, and when they began returning to spawn naturally in year 31, they mated primarily with themselves, because they were much more numerous than the wild fish (for the conditions stipulated, approximately 2500 returning hatchery fish in year 31 compared with 50 wild fish; see Table 2). These matings in the wild in year 31 thus produced inbred progeny, which began to return in year 34.

Under the increase scenario, the ≈2500 spawners in year 31 produced an equal number of fish returning in subsequent years, so a considerable number of inbred progeny returned in year 34, leading to an increase in IBD. In the crash scenario, the natural spawners in year 31 produced only 50 returning adults (spread among 3 yr), so the few inbred fish returning in year 34 had little effect compared to the large number of returning, noninbred hatchery fish (≈5000 per year). This swamping by returning, noninbred hatchery fish in the crash scenario continued until year 38, when inbred progeny resulting from the captive broodstock program were not offset by any noninbred hatchery fish. These inbred fish were derived from adults collected in year 29 that produced 4-yr-old captive fish in year 33, which in turn produced 5-yr-old returning adults in year 38.

Before reporting the genetic effects of variations to the standard model, we will briefly discuss other genetic parameters that were monitored in the simulations (R.S. Waples

TABLE 3. Cumulative increases in identity by descent (ΔIBD) relative to the control (no captive program) for simulations using a variety of captive broodstock scenarios. Only changes from the standard model are indicated. Parenthetical numbers are single generation effective population sizes (N_e) that would be expected to produce the same magnitude of increase in inbreeding. See Table 1 for variable definitions.

	N	NH	CP	HP	Other	ΔIBD (N_e)	
Standard	50	10	1,000	2	As in Table 1	0.0198 (22)	
Change	—	4	2,500	—	—	0.0623 (65)	
from	—	25	400	—	—	0.0051 (65)	
standard	100	—	—	—	—	0.0211 (22)	
	100	20	—	—	—	0.0091 (48)	
	200	—	—	—	—	0.0192 (25)	
	200	40	—	—	—	0.0049 (90)	
	—	—	—	10	—	0.0247 (18)	
	—	—	—	1	—	0.0168 (26)	
	—	—	500	—	—	0.0174 (25)	
	—	—	200	—	—	0.0132 (32)	
	—	—	100	—	—	0.0098 (40)	
							B-Holt Ricker
	—	—	—	—	Density dependence: $\alpha = 2$	0.0150 (28)	0.0739 (7)
					$\alpha = 4$	0.0184 (24)	0.1271 (4)
					$\alpha = 6$	0.0200 (22)	0.1429 (3)
	—	—	—	—	Random mating in hatchery	0.0198 (22)	
					Alternate-year mating	0.0206 (22)	
					Sex ratio equal in broodstock collected from wild	0.0164 (26)	
					Binomial variance in progeny number for broodstock collected from wild	0.0213 (21)	
	—	—	—	—	Proportion maturing by age (same in hatchery and wild):		
					3 4 5		
					0.25 0.50 0.25	0.0174 (25)	
					0.33 0.34 0.33	0.0155 (28)	
					0.33 0.34 0.33	0.0122 (34)[a]	

[a]This simulation was used to check for agreement with the Ryman and Laikre (1991) formula for computing N_e for a hatchery–wild system as a whole (Fig. 6; see text for discussion). Other departures from the standard model were an equal sex ratio and a binomial distribution of progeny number for broodstock collected from wild, and random mating of their progeny in the hatchery.

and C. Do, Northwest Fisheries Science Center, 2725 Montlake Boulevard E., Seattle, WA 98112, unpublished data). In general, these parameters showed a pattern of response similar to that for IBD. For example, the decline in heterozygosity was more rapid for a period following supplementation, after which the decline leveled off (in the increase scenario) or paralleled that in the control population (in the crash scenario). The variance in allele frequency over time followed a pattern similar to that for IBD, except that the effect was seen one generation earlier. The earlier increase in variance was expected, as changes in allele frequency occur in a single generation, whereas increases in IBD require two generations. We also examined the probability of loss of rare alleles from the population (defined as absence of the allele in M successive years of spawners). As expected, the "crash" scenario led to a much higher rate of allelic extinction than was found under either the control or the increase scenarios.

Results described above showed that the much larger population sizes that occur under the increase scenario stabilize these genetic parameters in the population. However, the consequences for the population were less sanguine when the increased abundance was not sustained. This crash scenario must be taken seriously because, for most of this century, artificial propagation has been used to mitigate factors for population decline rather than fixing them. Supplementation in general, and captive broodstocks in particular, are essentially unproven as a means of permanently increasing the abundance of Pacific salmon populations. Consequently, in the remainder of this study we focussed on the genetic consequences of failure to maintain artificially increased abundance.

To aid in summarizing results of a large number of simulations, we compiled data (see Table 3) that show the overall increase in IBD for a number of scenarios compared with that expected from a control run (no supplementation) with the same value of N. We refer to this overall increase in IBD as ΔIBD. Numerically, ΔIBD for a given year is simply the difference in IBD between the simulation under consideration and the control. To smooth out minor year-to-year fluctuations in simulation results due to random and age-structure effects, we computed ΔIBD as the average

difference in IBD with respect to the control over the last 15 yr of the simulation (i.e., years 61–75). For the range of parameters we considered, this approach was reasonable because the increase in IBD with time was nearly linear (with parallel slopes) for both crash and control simulations.

Density Dependence

In the crash scenario described above, the population declined precipitously. To evaluate the importance of the rate of the decline to presupplementation levels, we conducted simulations using both Beverton–Holt and Ricker recruitment functions for the population. These functions took the following forms:

$$(1)\quad N_{t+1} = \alpha \frac{N_t}{1 + \beta N_t},\ K = \frac{\alpha - 1}{\beta}\ \text{(Beverton – Holt)};$$

$$(2)\quad N_{t+1} = \alpha N_t e^{-\beta N_t},\ K = \frac{\log \alpha}{\beta}\ \text{(Ricker)}.$$

In the above formulas, K is the equilibrium population size, α represents the population growth rate with no density dependence, and β is a density-dependence scaling factor chosen to yield the desired value of K for a given value of α. In the analysis of density dependence, equilibrium population size was set at $K = N = 50$, and α values of 2, 4, and 6 were evaluated. Values in this range have been proposed for some stocks of Pacific salmon (e.g., Van Hyning 1973).

Simulations using Beverton–Holt recruitment functions produced overall levels of inbreeding that did not differ substantially from those found under the crash scenario (Table 3). The rate of population decline under Beverton–Holt recruitment was only slightly less rapid than under the crash scenario, leading to relatively small reductions in ΔIBD. For example, with $\alpha = 2$ under Beverton–Holt density dependence, ΔIBD was 0.0150 rather than 0.0198 under the standard model (Table 3). With $\alpha = 4$ or 6, however, the decline was nearly as rapid as in the crash scenario.

Results for the models using Ricker recruitment functions were quite different. Whereas a population that is above the equilibrium size and follows a Beverton–Holt recruitment function will approach the equilibrium point from above without ever dropping below K, the abundance of populations that follow Ricker recruitment functions may fluctuate wildly about the point of equilibrium. With an equilibrium point at $N = K = 50$ and α values of 2, 4, and 6, the Ricker model predicts essentially zero recruitment from spawning populations of 556, 278, and 220 fish, respectively. That is, if K is low and recruitment is determined by Ricker-type functions, a successful supplementation program could in theory drive the natural population to extinction. A similar phenomenon was discussed by Fagen and Smoker (1989). In the simulations using Ricker recruitment functions, escapements of zero fish were found in many of the years immediately following cessation of supplementation. In most replicates, the duration of zero escapements was generally less than M years, so the populations as a whole did not go extinct, but it was driven to very low levels from which recovery was slow. As a consequence, the cumulative effects of inbreeding were considerably greater than were found in the crash scenario (Table 3).

Because differences between results for the crash scenario and those using Beverton–Holt recruitment functions were relatively small, the remaining analyses considered only the crash scenario.

Scale of the Captive Propagation Program

Because the general problem considered here is the consequence of augmenting only part of the gene pool of a population, the scale of the captive propagation program is an important consideration. We examined this aspect of the problem in several ways. First, we considered a program with a fixed capacity for rearing adults to maturity but with three options for broodstock collection, spawning, and rearing. The three scenarios involved the following combinations of NH (number of adults taken for broodstock) and CP (mean number of progeny per female that were reared to maturity in captivity): 4, 2500; 10, 1000; and 25, 400. These scenarios all produced an average of 5000 adults per year, assuming an equal sex ratio. Data in Table 3 show that cumulative levels of inbreeding were much higher for the scenarios involving fewer initial adults per year. In particular, inbreeding increased quite substantially in the scenario using only four fish per year in the initial broodstock collections. Conversely, ΔIBD was relatively small if as many as 25 adults per year were taken for broodstock.

Next, we compared results for the following set of simulations (values are N, NH; CP was fixed at 1000 and HP at 2): 50, 10; 100, 20; 200, 10; 100, 20; 200, 40. These parameter sets were chosen to evaluate which is more important in determining overall levels of inbreeding: the absolute number of fish taken for broodstock or the proportion of the population sampled. As data in Table 3 illustrate, the magnitude of increase in IBD was nearly the same for all simulations using NH = 10 fish per year, regardless of whether total population size was $N = 50$, 100, or 200. Conversely, when the proportion of the wild population taken for broodstock was held constant at 20% per year (parameter sets 50, 10; 100, 20; and 200, 40), the magnitude of increase in IBD decreased considerably as NH increased. Thus, for the range of values considered here, the absolute number of adults taken from the wild for broodstock (NH) had a stronger influence on ΔIBD than did the proportion of the population sampled.

Finally, we considered effects on IBD of variation in the total production from the captive broodstock program. Key variables were CP and HP. Initial and equilibrium population sizes were 50 and NH was 10 in each simulation. Values considered for CP and HP, respectively, were 1000, 10; 1000, 2 (the standard set); 1000, 1; 500, 2; 200, 2; and 100, 2. Results showed the reduced levels of cumulative IBD in programs of smaller scale (Table 3). The controlling factor appeared to be the number of returning hatchery fish in the second generation that were derived from each female initially captured from the wild; that is, the product CP × HP was more significant than either CP or HP individually. For example, the overall increase in IBD in a captive broodstock program with parameters CP = 1000 and HP = 1 (.0168) was nearly the same as that for a program with CP = 500 and HP = 2 (0.0174). As expected, a successful smolt program (HP = 10 returning progeny per hatchery female) accelerated the rate of increase in inbreeding under the crash scenario (ΔIBD = 0.0247).

Program Duration and Marking Rate of Hatchery Fish

Intuitively, the duration of a captive broodstock program should be an important determinant of the cumulative increase in inbreeding experienced by the population. This should be particularly true if hatchery fish are unmarked, because

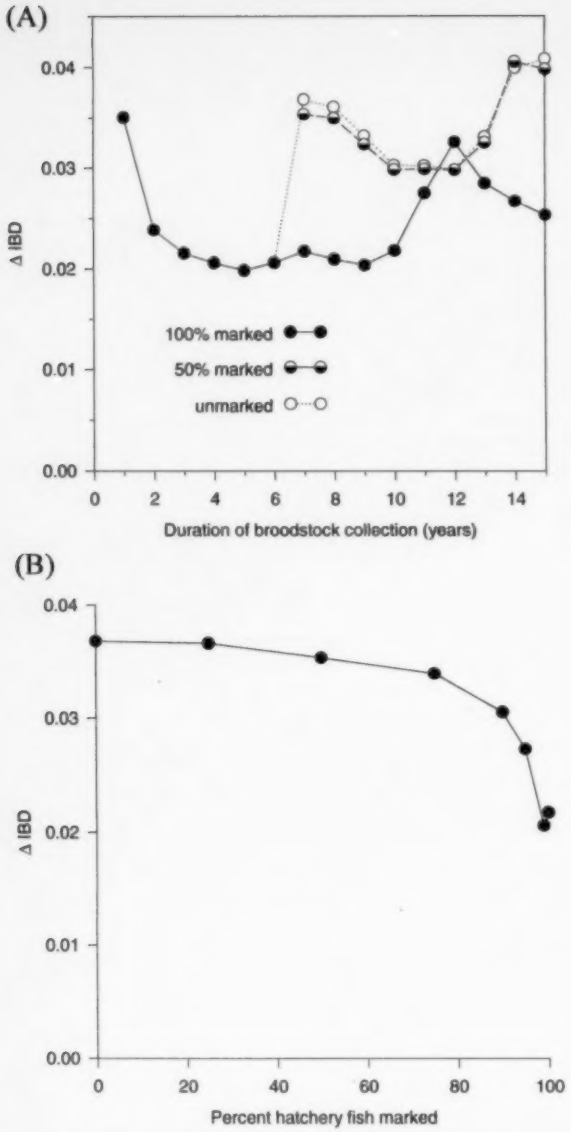

FIG. 3. (A) Cumulative increases in IBD (ΔIBD) attributable to captive broodstock programs of variable duration and with variable proportion of hatchery fish marked. Marked hatchery fish were not used for broodstock in subsequent years. Other parameters were as shown in Table 1. (B) ΔIBD for a program taking broodstock in seven consecutive years with a range of marking intensities.

such fish may be subject to broodstock collection when they return as adults, thereby leading to repeated enhancement of the same limited segment of the overall gene pool. Because returning adults from a successful captive broodstock program generally will greatly outnumber those produced naturally, random collection of unmarked fish for broodstock would yield primarily hatchery fish.

Table 3 and Fig. 3 show results of a series of simulations designed to evaluate the joint effects of program duration and identifiability of hatchery fish. Broodstock were collected for periods ranging from 1 to 15 yr. In any given simulation, the proportion of hatchery fish marked was 0, 0.5, or 1, and marked hatchery fish were not used for broodstock if they returned as adults.

Consider first results for simulations involving broodstock programs that lasted six or fewer years (i.e., with broodstock collection terminating in year 30 or earlier; see Table 3). In the standard model, the first hatchery fish did not return until year 31, so results for these simulations were identical for marked and unmarked fish. Interestingly, the cumulative effects of inbreeding were higher for programs of short duration. In particular, ΔIBD was greatest (0.0350) when broodstock was collected in only a single year. This result is due to a type of second-level Ryman–Laikre effect. That is, whereas Ryman and Laikre (1991) pointed out the genetic consequences of augmenting only part of a population's gene pool in a single generation, the effect is even more pronounced if individuals from only part of a generation are taken into the hatchery. In a successful captive broodstock program (as modelled here), even a single year of broodstock collection may provide enough offspring to overwhelm the wild population with fish derived from only a few individuals (in this case, 10 fish taken in year 25). Taking broodstock from additional years broadens the genetic base upon which the future population depends, thus reducing ΔIBD.

For programs with broodstock collection continuing until year 31 (i.e., those lasting 7 yr or more), marking of hatchery fish can make a substantial difference in ΔIBD (Fig. 3). In the standard model, about 99% of the fish returning in years 31 and later were hatchery fish. Thus, if the hatchery fish were not identifiable, broodstock taken in years 31 and following consisted almost entirely of unmarked hatchery fish. Because these unmarked hatchery fish were produced in the captive broodstock program, using them for broodstock initiates a second generation of amplifying a small part of the gene pool. For the simulation lasting 7 yr with unmarked fish, ΔIBD was particularly large (0.0368) because only a small portion of the original gene pool (all derived from broodstock collected in year 25) was being amplified a second time. Programs using unmarked fish and lasting 8–13 yr showed slightly lower levels of ΔIBD because a larger portion of the gene pool was being incorporated into the second round of captive propagation. The spike in ΔIBD seen in programs lasting 14 or 15 years signals the beginning of a third round of amplifying the same part of the original gene pool through captive propagation.

In contrast, programs that marked all hatchery fish and avoided using them for broodstock did not show an appreciable increase in ΔIBD unless broodstock collection lasted longer than 10 yr. Avoiding marked fish allowed augmentation of a different portion of the gene pool through captive propagation, and (at least for the parameter set considered here) this factor apparently was sufficient to temporarily postpone any further increase in inbreeding. Inevitably, however, successful programs that continue taking broodstock over a long period of time will reach the point at which unmarked fish taken for broodstock include progeny of naturally spawning hatchery fish derived from earlier years of the program. In our simulations, this effect was seen beginning in year 11 of broodstock collection (i.e., year 35; Fig. 3A). Most unmarked fish returning in year 35 were progeny of hatchery fish that spawned naturally in years 31

and 32, which in turn were derived from a relatively few individuals taken into captivity in the first year or two of the program. Thus, although 100% marking can postpone further increases in inbreeding due to a second round of amplifying the same portion of the gene pool, it cannot prevent such increases in programs that last long enough. In general, 100% marking can avoid further increases in inbreeding if broodstock collection continues for less than (X + Y + Z) years, where X, Y, and Z are the minimum ages of reproduction for captively reared, hatchery-released, and natural fish, respectively.

Intuitively, one might expect that marking 50% of the hatchery fish would be half as effective in reducing ΔIBD as marking all hatchery fish. However, this was not the case in the simulations; in fact, marking half the fish was little better at reducing ΔIBD than marking no fish at all (Fig. 3). The explanation for this result is simple. Under the conditions modeled here, the captive broodstock program was very successful and produced about 99% of the fish returning to spawn in years 31 and following. Even if half the hatchery fish are marked, unmarked hatchery fish still greatly outnumber returning natural fish, so broodstock collection will yield primarily unmarked hatchery fish. As shown in Fig. 3B, marking only began to be effective in reducing ΔIBD when over 90% of hatchery fish were marked. Benefits in reducing ΔIBD can be expected at lower marking rates in programs that are less successful in "swamping" the wild population with fish derived from the supplementation program.

Mating and Rearing Protocols

A considerable amount has been written about how different mating strategies can influence effective population size and the rate of loss of genetic variability in Pacific salmon. Some practices are widely recognized as having deleterious genetic effects (although this recognition does not always translate into appropriate action in practice). For example, fertilizing more than one female with the same male, combining gametes from multiple individuals prior to fertilization, and exporting progeny of entire families to other facilities have all been shown to reduce genetic variability (Gharrett and Shirley 1985; Simon et al. 1986; Allendorf and Ryman 1987; Withler 1988).

In our models, we assumed that these gross malpractices were avoided, and we considered instead different mating strategies within a captive broodstock program. Because a captive broodstock program involves rearing fish to maturity and spawning them, there is the opportunity to determine at least a one-generation pedigree for the broodstock, and that knowledge can be used to design specific mating strategies. With respect to the model used here, the question thus becomes, What mating strategies will minimize the cumulative ΔIBD attributable to captive propagation?

In the standard model, we simulated sib-avoidance matings in the hatchery to the maximum extent possible. This meant that when each captively reared individual matured, it was mated with another individual that was not a full or half sibling unless no other mates were available. In most simulations, sib matings were avoided approximately 97–99% of the time. In an actual captive broodstock program, a similar level of sib-avoidance could be achieved by keeping all different family groups separate throughout captivity, or at least until the stage at which they can be marked so that they can be identified prior to spawning.

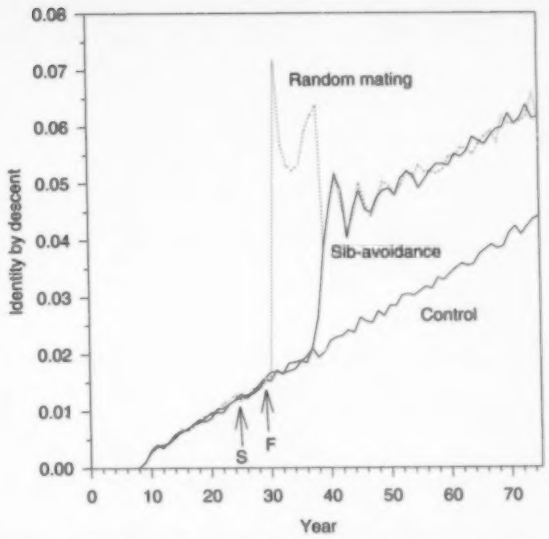

FIG. 4. Comparison of pattern of increase in IBD for simulations in which random and sib-avoidance matings were used with captively reared fish that spawned in the hatchery. Other parameters were as shown in Table 1. S and F indicate the start and finish, respectively, of broodstock collection.

Neither of these scenarios is technologically difficult, but either might prove difficult or impossible to achieve in some facilities. Therefore, we also considered the consequences of ignoring pedigree entirely and allowing random mating among all captively reared fish maturing in a given year. Results for this model compared with the standard sib-avoidance model are shown in Fig. 4 and Table 3. With random mating, there was a large spike in IBD that occurred in year 31, when the first progeny of the captive broodstock program returned as adults to spawn naturally. These were derived from the first fish to mature in captivity (in year 28), which in turn were derived from only 10 wild adults collected for broodstock in year 25. With random mating in the hatchery in year 28, a substantial proportion of full- and half-sib matings occurred, which led to an increase in IBD when these fish returned to spawn naturally in year 31. As noted above, with sib-avoidance mating practiced in the hatchery, the increase in IBD was postponed until year 38.

From about year 40 on, however, there was no difference in IBD between the scenarios involving random and sib-avoidance mating (overall ΔIBD = 0.0198 for both scenarios; Table 3). This result illustrates the common maxim that inbreeding changes genotypic frequencies but not allele frequencies. That is, although sib-avoidance mating postpones the point at which alleles that are identical by descent unite within individuals, sib-avoidance by itself does not alter the frequencies of these alleles in the population. Eventually, with restoration of random mating in the wild, IBD will equilibrate at a point that reflects the overall frequencies of the alleles remaining in the population. Thus, sib-avoidance mating had no permanent effect on the level of inbreeding.

It should be noted, however, that this result depends on the assumption that all alleles are selectively neutral; a different pattern may be found if selection is involved. In particular, a temporary increase in IBD (as may occur in the hatchery

with random mating) may result in an increase in the proportion of individuals that are homozygous for deleterious, recessive alleles. The reduction in fitness that can accompany this increase in homozygosity is known as inbreeding depression and can have a permanent effect on a population's fitness. Alternatively, because higher levels of inbreeding provide greater exposure of deleterious alleles to natural selection, it is possible that a brief episode of inbreeding will help to purge deleterious alleles from a population, thus leading to higher fitness. This study did not attempt to evaluate the possible fitness effects of increased levels of inbreeding.

Another sib-avoidance strategy is to mate individuals from different brood years whenever possible. Because Pacific salmon are semelparous (invariably dying after spawning), individuals of different ages cannot be siblings. Keeping progeny from each brood year separate in captivity is generally easier than separately rearing each family group within years. In fact, most hatcheries rearing multiple brood years of fish keep them separate at least through the first 2 yr.

In the alternate-year mating strategy we considered, pairwise matings were conducted with individuals of different ages until only one age group remained, at which point matings within years were allowed. For example, if 1000 age-4 fish and 3000 age-3 fish matured in captivity in a given year and sex ratio was equal, all age-4 fish would mate with age-3 fish. The remaining 2000 age-3 fish would mate among themselves, with sib-avoidance practiced within years to the extent possible. Results in Table 3 show that ΔIBD for the alternate-year mating strategy (0.0206) was similar to the value for sib-avoidance mating under the standard model (0.0198). There are other variations of the alternate-year mating strategy, and their effects on the overall level of inbreeding can be complex and are sensitive to the age structure of the population (R.S. Waples and C. Do, Northwest Fisheries Science Center, 2725 Montlake Boulevard E., Seattle, WA 98112, unpublished data).

Another aspect of mating protocols that may be under control in the hatchery is the sex ratio of fish captured from the wild. In the standard model, sex ratio varied randomly. In a simulation in which sex ratio was held constant at five males : five females, ΔIBD was smaller than under the standard model (0.0164 vs. 0.0198). This reduction in cumulative levels of inbreeding reflects an increase in N_b in the hatchery, which results from using the same number of each sex. Clearly, this benefits the program, particularly if NH is small, in which case the proportional reduction in N_b due to random fluctuations in sex ratio may be substantial. However, if a large fraction of the population is taken into captivity, forcing an equal sex ratio in the hatchery may result in an unacceptably skewed sex ratio (and reduced N_b) in the remaining natural population.

Age Structure Effects

With their combination of semelparity and overlapping year classes, Pacific salmon have one of the most challenging life-history patterns to model mathematically. Waples (1990*a*) showed that, with appropriate adjustments, effective population size and the rate of loss of genetic variability in Pacific salmon can be understood in terms of standard population genetic theory based on organisms having discrete generations. However, the model he considered involved a single, closed population, and his results by no means indicate that the complexities of salmon life history can be ignored in all cases. Some of our results (e.g., the strong dependence of ΔIBD on the exact number of years of broodstock collection and the different timing of increases in IBD under different mating scenarios) suggest the importance of age structure in determining the genetic consequences of captive broodstock programs. In this section, we explore this topic in more detail.

First, we examined what would happen if all fish, whether naturally spawned, captively reared, or hatchery released, had the same age structure (25% age 3 and 5 and 50% age 4). This contrasts with the standard model, in which captively reared and hatchery-released fish matured at an earlier average age. The resulting value for ΔIBD (0.0174; Table 3) was somewhat lower than that obtained under the standard model (0.0198). This reduction in ΔIBD can be attributed to two factors. In the standard model, early maturing hatchery fish reduced the generation length, and this shorter generation length allowed for more genetic drift (and inbreeding) to occur over a fixed number of years. We believe this was a minor effect because only about two generations were directly affected by the hatchery program. More importantly, the age structure used in the standard model for hatchery and captively reared fish was heavily skewed toward age-3 fish, in contrast to the more even age distribution in the wild. Changing to the same age distribution in the hatchery and in the wild reduced the possibility for individual age classes to contribute disproportionally to later generations. The importance of this latter factor is illustrated by results of another simulation, in which one third of the fish matured at ages 3, 4, and 5 in both the hatchery and the wild. With equal proportions of each age, ΔIBD was 0.0155, a further reduction from that found with age structure of 25, 50, and 25% for 3-, 4-, and 5-yr-old fish, respectively.

Second, to examine the effects of age structure more carefully, we ran a series of simulations using the standard model, with each allele in the total population in years 25–29 assigned a unique value. This amounted to reinitializing the population in a fashion similar to that done for years 1–5. This recoding allowed us to trace each allele found in the population in subsequent years back to the brood year from which it originated. Furthermore, alleles that remained in the wild population (≈40 fish per year) were monitored separately from those that were taken to the hatchery (10 fish per year). Results of these simulations are revealing on two counts.

First, in both the crash and increase scenarios, virtually all alleles remaining in the population at year 75 (99.85%) were derived from the 50 total fish taken into the hatchery in years 25–29. Swamping of the wild contribution (0.15%) was almost complete. Second, under the crash scenario, the genetic contribution to future generations of fish taken as broodstock in years 25–29 was highly skewed to favor later years (Fig. 5). Broodstock from a single year (year 29) were responsible for over 60% of the total alleles remaining in the population, and years 28 and 29 together were responsible for over 85% of the alleles. Conversely, fish collected in year 25 made virtually no permanent genetic contribution to the population, and those collected in year 26 made very little. This phenomenon was limited to the crash scenario, as representation of the different brood years was even in the increase scenario (Fig. 5).

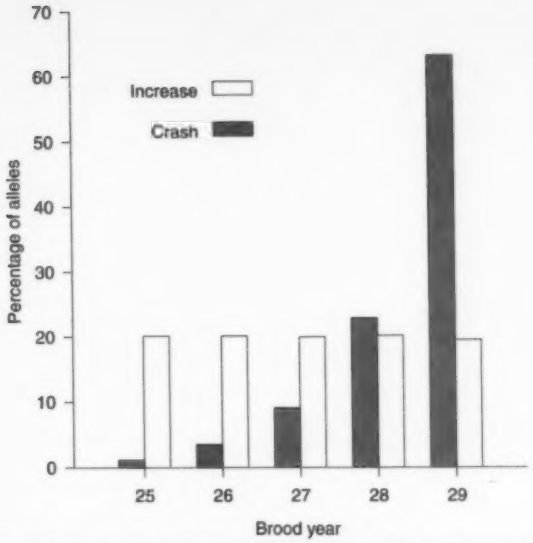

FIG. 5. Proportion of alleles remaining in population in year 75 that originated in each of the 5 yr of broodstock collection (years 25–29). Results are shown for both crash and increase scenarios. Other parameters were those of the standard model (Table 1).

This disproportionate representation of later brood years under the crash scenario can be understood by examining data shown in Table 2. Progeny of adults collected in year 25 matured in captivity in years 28 and 29, and their progeny returned to spawn naturally in years 31–34. Although hatchery fish returning in years 31–34 numbered in the thousands, naturally spawning fish in each of these years produced only 50 fish that returned to spawn in subsequent years. The genetic contribution of these 50 fish, in turn, was swamped by the much more numerous hatchery fish returning each year through year 38. Thus, the genetic contribution of broodstock collected in year 25 was essentially nullified under the crash scenario, and a similar (albeit not so drastic) phenomenon applied to broodstock collected in years 26–28. In contrast, all hatchery fish returning in the last year of hatchery returns (year 38) were derived from adults collected in year 29, and this brood year therefore predominated in the final genetic makeup of the population.

Effective Size of Individual Hatchery and Wild Populations

In discussing the consequences of supportive breeding for genetic diversity, Ryman and Laikre (1991) provided the following formula for computing the effective size (N_e) of a captive–wild system as a whole:

$$(3)\quad \frac{1}{N_e} = \frac{f_c^2}{N_{ec}} + \frac{f_w^2}{N_{ew}},$$

where f is a proportion of the total population and the subscripts c and w refer to captive and wild, respectively. Equation 3 shows that the effective size of the larger system is a function of the effective sizes of both the captive and wild phases individually.

Intuitively, a key factor in determining N_e for the system as a whole should be the difference between the effective size of broodstock taken into the hatchery and the effective size if the fish had been left to spawn in the wild. Captive spawning and rearing affords the opportunity to reduce the variance in the number of progeny per parent that survive to reproduce (V_k) and, in theory, increase the effective size in captivity. For example, in an ideal population (with equal sex ratio and V_k = binomial (random)), $N_e = N$. It is well known that if V_k is less than the binomial variance, N_e can be larger than N, and if $V_k = 0$ (all individuals having the same number of progeny), N_e can be almost twice N. Because the standard model assumed $V_k = 0$ in the captive-rearing phase, we also wanted to consider the consequences of not being able to equalize progeny number this effectively. In a simulation using the standard model, except that the distribution of progeny in the captive phase was binomial, ΔIBD was 0.0213 (Table 3). Surprisingly, this was only slightly higher than the value found under the standard model (0.0198) with $V_k = 0$.

Why did equalizing progeny number in the captive phase have relatively little effect on ΔIBD if doing so should nearly double the effective number of breeders? The answer can be found by examining the formulae for computing inbreeding (N_{ei}) and variance (N_{ev}) effective population size as a function of the number of spawning adults in the parental generation (N) and the mean ($\bar{k}$) and variance (V_k) of the number of surviving progeny per parent:

$$(4)\quad N_{ei} = \frac{N\bar{k} - 2}{(\bar{k} - 1) + V_k/\bar{k}}$$

(Crow and Kimura 1970, Equation 7.6.2.17)

$$(5)\quad N_{ev} = \frac{N\bar{k} - \bar{k}}{1 + V_k/\bar{k}}$$

(Crow and Kimura 1970, p. 359).

Assuming an equal sex ratio and constant population size ($\bar{k} = 2$), both the inbreeding and variance effective numbers reduce to $N_e = 2N - 2$ when $V_k = 0$. However, this rule of thumb does not necessarily hold if population size is changing. In particular, if $\bar{k}$ is large and the population is rapidly increasing (as will be the case in a successful captive broodstock program), the value of V_k is of little importance in determining N_{ei} (see Equation 4).

This point is illustrated in Table 4, which shows the computation of inbreeding and variance effective sizes for several stages before, during, and after implementing a captive broodstock program. The values shown assume that sex ratio is equal and the distribution of progeny number is binomial. For the captive-rearing phase, the mean number of surviving progeny per parent was large ($\bar{k} = 1000$) and this term dominated the denominator of the formula for N_{bi}. Thus, as $\bar{k} \to \infty$, $N_{bi} \to N$ regardless of V_k, and equalizing progeny number has little effect on N_{bi} during a population increase. During the increase phase, the variance effective number did nearly double (from 5000 to 9000) when V_k was reduced from the binomial variance to 0. However, because essentially no genetic drift or inbreeding occurs over a single generation in a population with an effective size of 5000, nearly doubling N_{ev} in the captive phase would have little genetic effect on the population.

However, reducing V_k below the binomial variance can have a substantial effect on N_{bi} in a declining population. Considering only the captive phase, selection of just NH = 10 adults for the captive broodstock program from the 50

TABLE 4. Inbreeding and variance effective population numbers at several stages before, during, and after implementing a captive broodstock program. Values shown were obtained using Equations 4 and 5 in the text. To simplify this illustration, an equal sex ratio was assumed and age structure was ignored. For each phase, N_1 is the population size in the adult generation and N_2 is the population size in the progeny generation. k and V_k are the mean and variance of the number of surviving offspring per spawning adult. Some V_k values have been rounded off. Effective population numbers are shown for the case of a fixed number of offspring per individual ($V_k = 0$) and random variance in progeny number (V_k = Binom).[a] The pattern of population growth and decline is the same as occurs under the standard model (see Table 2).

Phase	N_1	N_2	$\bar{k}$	Inbreeding effective size (N_{ei})[a]		Variance effective size (N_{ev})	
				V_k = Binom	$V_k = 0$	V_k = Binom	$V_k = 0$
Natural production	50	50	2	50	98	50	98
Broodstock collection	50	10	0.4	47	∞[b]	10	12[b]
Captive rearing	10	5000	1000	10	10	5000	9000
Hatchery release	5000	5000	2	4999	9998	5000	9998
Population crash	5050[c]	50	0.02	4999	∞[b]	50	50[b]
Natural production	50	50	2	50	98	50	98
Harmonic mean over entire cycle				37	50	37	48

[a]If the sex ratio is equal and the distribution of progeny number is random, $V_k = \bar{k}(1 - 1/N_1)$ (Crow and Kimura 1970, Equation 7.6.2.4).

[b]V_k cannot be 0 unless k is an integer, so the minimum possible variance (see text for explanation) was used in computing N_e.

[c]Includes 50 naturally produced fish.

total adults in the population represented a sharp reduction in the population. In this case, the mean number of progeny per adult was small ($\bar{k} = 0.4$), and the value of V_k assumed greater importance in determining N_{bi}.[1] For example, if V_k during this phase could be reduced from 0.392 (binomial variance) to 0.316 (a 20% reduction), then N_{bi} would be doubled from 47.4 to 94.8. In general, however, there is no way to control V_k during this phase because broodstock collected from the wild would be of unknown parentage.

As shown in Table 4, inbreeding and variance effective sizes were the same during periods of population stability but differed during changes in population size. These differences reflect the fact that N_{ev} is determined primarily by the number in the progeny generation and N_{ei} is determined primarily by the number in the parental generation. Therefore, changes in the variance effective number appear one generation before they do for the inbreeding effective number. Over the entire increase–crash cycle, however, the two effective numbers were essentially the same (Table 4). Behavior of the two effective numbers under a supportive breeding program is also discussed by Ryman (1994).

Thus far we have considered only situations in which V_k for both the captive and wild phases is less than or equal to the binomial variance (resulting in $N_b \geq N$). This is probably an unrealistically high value for N_b. In natural populations, the effective size is generally less than N, and often a great deal less. There are few data on the ratio N_b/N for Pacific salmon, but Waples et al. (1993) used indirect genetic methods to obtain estimates of N_b/N in the range of 0.2–0.7 for several populations of Snake River chinook salmon. Similarly, factors such as prespawning mortality after collection and asynchronous maturation of adults collected for broodstock can substantially reduce the number of fish that are available for spawning. Female fecundities and sperm viability may also vary considerably, confounding attempts to equalize the number of progeny from captive individuals. In a captive broodstock program for threatened winter-run chinook salmon at Coleman Hatchery, Hedrick (1994) estimated that, because of a combination of factors, collection of 23, 29, and 18 adults in 1991, 1992, and 1993, respectively, resulted in N_b values of only 5.9, 17.9, and 7.8. This is a mean N_b/N of only 0.44 (range 0.26–0.62).

To evaluate the consequences of an effective size less than N, we conducted a series of simulations in which N_b/N took values of 0.2, 0.4, 0.6, 0.8, and 1.0 in the captive and wild independently. The value of N_b was reduced to the desired fraction of N by forcing an equal sex ratio and spawning only a fraction N_b/N of the captive or wild fish, with binomial variance in progeny number. So that population size was not affected by spawning only a fraction of the individuals, the total number of offspring produced remained the same as would have occurred by spawning all N fish. A third variable was the effective size for the generation of captively reared fish that matured in the hatchery. In the standard model, progeny of these fish were released to the wild and returned at a replacement level (mean of two per female) with binomial variance. Under these conditions, N_b was $N - 1$. For the new simulations, we fixed N_b/N for these captively reared fish at 0.6, using the same procedure described above. Use of $N_b/N = 0.6$ was arbitrary but not unreasonable, and it allowed us to consider only a two-dimensional matrix of N_b/N values for the captive and wild populations. In practice, the exact value of N_b/N for the captively reared phase mattered little because the total number

[1]Because reducing V_k to 0 requires that all individuals leave the same number of offspring, and that can only occur if all individuals leave exactly $\bar{k}$ offspring, V_k cannot be 0 unless $\bar{k}$ is an integer. Thus, in rapidly declining populations ($\bar{k} < 1$), it will be impossible to reduce V_k to 0. The best that could be achieved would be to have each of the gametes in the progeny generation derived from a different individual in the parental generation. This scenario results in the "minimum variance" used in Table 5.

TABLE 5. Cumulative increases in IBD (ΔIBD) resulting from captive broodstock programs with various ratios of N_b/N in the captive and wild phases. N_b/N for the hatchery-release phase was set at 0.6. The standard parameter set was used except for adjustments to yield desired N_b/N ratios (see text for explanation).

	Wild N_b/N				
Captive N_b/N	0.2	0.4	0.6	0.8	1
0.2	0.0684	0.0913	0.0988	0.1033	0.1084
0.4	0.0212	0.0399	0.0467	0.0483	0.0501
0.6	0.0043	0.0210	0.0286	0.0287	0.0319
0.8	−0.0003	0.0132	0.0193	0.0210	0.0212
1	−0.0023	0.0080	0.0128	0.0147	0.0168

of fish maturing in captivity was generally several thousand. Thus, even with a low N_b/N ratio, the amount of inbreeding occurring in this phase would have been negligible compared with that occurring in the captive and wild phases (see Table 4).

Results of the new simulations are shown in Table 5, which gives ΔIBD values compared with a control run (no hatchery) for each value of N_b/N in the wild. Most of the combinations of captive and wild N_b/N ratios resulted in ΔIBD values as large or larger than those found using the standard parameter set. In general, lower N_b/N ratios led to higher ΔIBD values, even when captive and wild N_b/N ratios were the same. For example, ΔIBD was 0.0168 with $N_{bc}/N = N_{bw}/N = 1$ but increased to 0.0684 with both captive and wild $N_b/N = 0.2$. Relatively low values of ΔIBD were found in some simulations in which captive N_b/N was larger than in the wild. In two runs, ΔIBD was essentially 0 (ΔIBD = −0.0023 for $N_{bc}/N = 1$ and $N_{bw}/N = 0.2$; ΔIBD = −0.0003 for $N_{bc}/N = 0.8$ and $N_{bw}/N = 0.2$). Thus, under some circumstances, a captive broodstock program may have essentially no permanent effect on the level of inbreeding, even under the crash scenario.

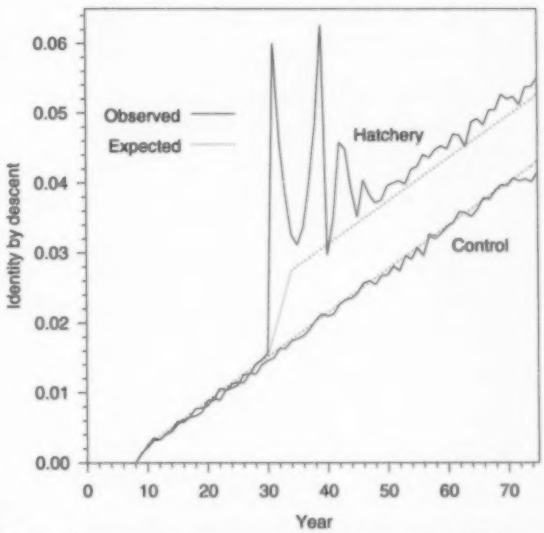

FIG. 6. Observed and expected pattern of increase in IBD over time for the control and crash scenarios with $N = 50$. The crash scenario followed the standard parameter set except that sex ratio was equal for broodstock collected from the wild, random mating was used in the hatchery, and age structure was equal at one third each for 3-, 4-, and 5-yr-old fish, both in the wild and in the hatchery. The expected pattern of increase in IBD in the crash scenario is based on Ryman and Laikre's (1991) formula (Equation 3) that predicts a one generation bottleneck of $N_e = 40$ for the parameters used in the simulation. The observed IBD is consistent with a single generation bottleneck of approximately 34 (see text for discussion).

Effective Population Size of the Captive–Wild System

As noted above, Ryman and Laikre's (1991) model did not consider age-structured populations and evaluated only a single episode of captive rearing. To determine whether Equation 3 can be used to predict the effective size of a captive–wild system of Pacific salmon, we considered a model in which as many attributes as possible were similar to those of the discrete generation model considered by Ryman and Laikre. Age structure was made equal at 33.3% for 3-, 4-, and 5-yr-old spawners, with no difference between hatchery, captive, and wild fish. Sex ratio was equal for broodstock collected from the wild, the number of offspring per individual was binomial rather than fixed, and random mating was used for captively reared fish. Other parameters were as in the standard model.

To compute the effective size of the captive–wild system using Equation 3, we first computed N_b values and then converted these to effective size per generation (N_e) using the relationship $N_e = gN_b$. Each year NH = 10 fish were taken into the hatchery and spawned. With an equal sex ratio and a binomial distribution of progeny number, N_{bc} = NH = 10 (Table 4). An average of 40 fish remained in the wild; with random mating and a random sex ratio, N_{bw} was approximately 40 − 1 = 39 per year. In subsequent years, returning progeny of fish collected for broodstock in years 25–29 outnumbered wild fish by approximately 5000:50, or 100:1. Thus, we have $f_c \approx 0.99$ and $f_w \approx 0.01$. Substituting these values in Equation 3 yields $1/N_b \approx 0.0995$, or $N_b \approx 10$, just the effective size of the captive phase alone. That is, swamping by hatchery fish essentially nullified the genetic contribution of the wild fish.

To compute the effective population size per generation, we multiplied the effective number per year by the average age at reproduction (4 yr): $N_e = 4 \times 10 = 40$. Thus, Ryman and Laikre's formula predicts that a supplementation program similar to that used in the standard model should result in a one-generation effective size for the captive–wild population as a whole of approximately 40.

A value of $N_e = 40$ represents a bottleneck, because the effective size per generation would have been $4 \times (N - 1) = 4 \times 49 = 196$ in the wild population alone without supplementation. Using the relationship that a one-generation change in IBD is approximately $1/(2N_e)$, we calculate that, according to Ryman and Laikre's formula, IBD should have increased by approximately $1/80 = 0.0125$ over one generation as a result of the captive broodstock program. This is considerably larger than the increase expected under the control scenario ($1/392 = 0.00255$). Figure 6 shows the pattern of increase in IBD in the control scenario (no supplementation) and that predicted by Ryman and Laikre's formula for the parameter values considered here.

The increase in IBD observed in the simulation was larger than that predicted by Equation 3, as shown in Fig. 6. Several factors might explain this difference. First, broodstock collection occurred for 5 yr (equal to the maximum age at reproduction), whereas a generation in the standard model was only 4 yr. However, as shown in Fig. 3A, ΔIBD was nearly the same for programs lasting either 4 or 5 yr, so this factor was apparently not important in this case. Second, although broodstock collection lasted for only about one generation, the progeny of fish originally collected for broodstock were reared and spawned in the hatchery, and the entire program, from initiation of broodstock collection in year 25 to the last returns of hatchery-reared fish in year 38, spanned about three salmon generations. However, the number of captively reared fish spawned each year in the hatchery ranged from about 1000 to about 5000 (Table 2), so little additional genetic drift would be expected from that phase of the program. Data presented in Table 4 show that almost all of the bottleneck effects can be attributed to the broodstock collection – captive rearing phase.

We believe that the effects of age structure depicted in Fig. 5 acted to reduce effective population size in the population as a whole beyond that predicted by Equation 3. The overrepresentation of later brood years means that there was a large variance in reproductive success (measured as eventual contribution to the population) among individuals from different brood years taken into the hatchery. However, this bias was not caused by events that occurred in the hatchery; rather, it resulted from the eclipse of production from the early years of the captive broodstock program by population crashes that occurred after the hatchery fish returned to spawn naturally. Equation 3, which only includes terms for effective size in the hatchery and wild components individually, does not account for this interaction between genetic and demographic parameters in age-structured populations and, therefore, leads to an overestimate of the size of the population bottleneck under the crash scenario. The discrepancy was not large for the simulation depicted in Fig. 6 because every effort was made to minimize age-structure effects. However, most of the other scenarios considered in Table 3 resulted in higher ΔIBD values, which would not be in such close agreement with predictions of Equation 3.

A convenient way to express the significance of ΔIBD values is to calculate the single generation N_e that would be expected to produce the observed level of increase in inbreeding. In a single generation, the total increase in IBD can be computed as the sum of two components: the amount IBD would increase under the control scenario, and the additional increase (ΔIBD) due to the captive broodstock program. As noted above, during a single generation, IBD would be expected to increase by 0.00255 in the control scenario when $N = 50$. For the simulations depicted in Fig. 6, ΔIBD was 0.0122 (Table 3). Therefore, the total increase in IBD that can be attributed to a single generation of a captive broodstock program was approximately $0.0122 + 0.0026 = 0.0148$. Based on the formula $\Delta IBD = 1/(2N_e)$, we calculate that the captive broodstock program under this scenario had a genetic effect on the population equivalent to a single-generation bottleneck of approximately $N_e = 34$, or somewhat less than the $N_e = 40$ predicted from Equation 3. For the standard model, with ΔIBD = 0.0198, the corresponding value is $N_e = 22$, or just over half that predicted by Ryman and Laikre's equation. Table 3 shows single generation N_e values that correspond to observed ΔIBD values for each simulation.

Discussion

Results of the simulations provide several important insights regarding the genetic consequences of using captive broodstock technology to supplement Pacific salmon populations.

Sustainability of Increased Abundance

The single most important factor determining the effects of a captive broodstock program on the level of inbreeding was whether the natural population remained large following termination of the program. This, in turn, will be determined largely by whether the factors responsible for the population's decline (or that are impeding recovery) have been adequately addressed. This result indicates that the philosophy that has guided artificial propagation of Pacific salmon for almost a century—that is, use of fish-culture techniques to mitigate (rather than fix) factors for decline, will not be sufficient to safeguard genetic resources of wild populations through supplementation by captive broodstocks. Only if we make substantial progress in addressing underlying causes of population decline concomitantly with (or in lieu of) initiating captive broodstock programs can we expect long-term, sustainable increases in population abundance.

However, our results also show that if recovery efforts are successful in increasing the productivity and/or the carrying capacity of the environment, then a captive broodstock program that leads to rapid population increase can actually result in lower overall levels of inbreeding than would occur without supplementation. In this increase scenario, there would be no genetic bottleneck and no loss of genetic diversity because hatchery-produced fish would truly supplement (rather than replace) natural fish. That is, the larger, post-supplementation population would contain all the genetic diversity that would have persisted in the absence of supplementation, as well as a great number of additional genes derived from the captive broodstock program.

Still, genotypic and allelic frequencies in the population would likely have changed from their presupplementation values, and a large fraction of the postsupplementation population (over 99% in our simulations) would be derived from the relatively few individuals taken into captivity. Furthermore, there might be adverse genetic effects owing either to the large, temporary spike in inbreeding caused by the captive broodstock program or to altered selective regimes

experienced in the captive environment. Therefore, although we can say that long-term increases in inbreeding are not a major concern in the increase scenario, sustained population increase is not a guarantee against other potential genetic problems associated with captive broodstock programs.

Much of this paper has focussed on the crash scenario, and we believe that this scenario should be considered seriously for several reasons. First, captive broodstocks are often proposed for populations that have reached low escapement levels but for which there are uncertain prospects for increasing productivity or carrying capacity. An example of this is the program under way for Dungeness River spring chinook salmon (J. Shaklee, Washington Department of Fisheries, P.O. Box 43151, Olympia, WA 98504, personal communication). After careful analysis of various enhancement strategies, the program was initiated in 1992 and is expected to last for 8–12 yr. However, it is acknowledged that success will depend on identification and correction of habitat and/or harvest constraints within that time. Second, even if there are reasonable prospects for improving habitat and good intentions of doing so, there is no guarantee that such efforts will produce the intended result. Finally, although it is well documented that hatcheries often (but not always) produce more returning adults than could be produced naturally, the use of hatcheries to permanently increase the abundance of natural populations has not yet been demonstrated to be a viable strategy (e.g., Miller et al. 1990). Therefore, the genetic risks associated with failure to sustain higher population levels must be evaluated at the time a captive broodstock program is considered.

Results of simulations that included density dependence should also be weighed carefully in assessing the consequences of the crash scenario. In particular, if Ricker spawner–recruit relationships apply and carrying capacity remains low, then a large, temporary increase in abundance caused by a captive broodstock program could theoretically drive the population to extinction.

Effective Size of Captive and Wild Populations

Results of the simulations suggest that, under the crash scenario, a captive broodstock program may have little permanent effect on the overall level of inbreeding in one situation: if N_b/N in the captive populations is much larger than in the wild. However, this phenomenon was only found in two simulations in which captive N_b/N was an optimistically high 0.8–1.0 and wild N_b/N was a relatively low 0.2. Most other combinations of captive and wild N_b/N ratios led to ΔIBD values as large or larger than those found under the standard model. Therefore, under the crash scenario, increased levels of inbreeding should be expected to result from a captive broodstock program unless N_b/N in the wild is known to be low and there are assurances that N_b/N in the captive phase will be close to 1.

Age Structure Effects

Simulation results showed that the pattern of semelparity with overlapping year-classes found in Pacific salmon can strongly affect the genetic consequences of captive broodstock programs in at least two ways.

First, cumulative levels of inbreeding were not a linear function of the duration of a captive broodstock program. Instead, they were strongly dependent on the exact number of years in which broodstock were collected. This means that the appropriate duration of a captive broodstock program should be evaluated on a case-by-case basis. In general, programs that collect broodstock from only part of a generation, or that last long enough to incorporate genes derived from earlier years of the program, will result in higher levels of inbreeding. Marking hatchery fish so that they can be avoided in collecting broodstock can postpone increases in inbreeding owing to repeated amplification of the same limited portion of the original gene pool. However, if the captive broodstock program leads to large (albeit temporary) increases in the abundance of naturally spawning fish, the benefits of marking in reducing inbreeding will be minimal unless the marking rate is nearly 100%.

Second, under the crash scenario, individual brood years can have an overwhelming influence on the future genetic makeup of a population. The exact nature of these effects can be quite complex and heavily dependent on the age structure of the population. These effects are a type of edge effect because they are primarily associated with the first and last years of broodstock collection. In normal supplementation programs of indefinite duration, these effects would be less important. In a captive broodstock program of short duration, however, these edge effects can be substantial. In particular, the latest maturing fish from the final year of hatchery releases can have a genetic effect much greater than their proportional representation would suggest. It is possible to develop mating protocols to minimize the effects of this phenomenon, but their effectiveness depends on accurate estimates (or predictions) of age structure for captive, hatchery, and wild fish (R.S. Waples and C. Do, Northwest Fisheries Science Center, 2725 Montlake Boulevard E., Seattle, WA 98112, unpublished data).

In evaluating age-structure effects, we have implicitly assumed that reproductive success is independent of age. In reality, this is unlikely to be true; older fish are generally larger, and this generally translates into more eggs for females and increased reproductive success for males, at least in the wild. Fortunately, age-specific reproductive success can be incorporated in our model in a relatively straightforward way. Instead of focussing simply on the proportion of fish maturing at each age, the relevant input parameters would be the proportion of offspring in each brood year produced by each age class. This can be obtained by weighting the proportion maturing at each age by age-specific reproductive success. For example, if the age structure of naturally spawning adults were one half age 4 and one quarter each age 3 and age 5 (as in the standard model), and if fecundity of 3-, 4-, and 5-year-old fish were 2000, 3000, and 4000, respectively, then it is easy to show that 5-year old fish would produce one third of the total offspring; 4-year old fish, one half; and 3-year-old fish, one sixth. Use of these latter values as input parameters for age structure in our model would provide a means of approximating the effects of age-specific reproductive success.

Scale of Program and Broodstock Collection

Choice of an appropriate scale for a captive broodstock program is an essential consideration, whose importance increases with uncertainty about the prospects for maintaining long-term abundance. In choosing the appropriate scale for a program, managers must simultaneously balance several different risks. On one hand, if short-term risk to a natural population is high, aggressive measures to build the popu-

lation size quickly may be called for, and taking a substantial fraction of the wild population for broodstock may offer the best prospects for accomplishing this. The Ryman–Laikre effect also argues for avoiding situations in which only a small fraction of the gene pool is enhanced at the expense of the remainder. On the other hand, concerns about directional genetic changes associated with captive rearing increase with the proportion of the population exposed to the captive environment. Similarly, risks to the remaining natural population from "mining" wild broodstock and the risk of catastrophic failure of cultured populations argue for limits on the proportion of a population that should be taken into captivity.

In extreme cases, the risk inherent in taking the entire population into captivity may be warranted. For example, this strategy is being used with Snake River sockeye salmon, an endangered species that has had adult returns of 4, 0, 4, 1, 8, and 1 fish over the period 1989–94 (Idaho Department of Fish and Game, 600 S. Walnut St., Boise, ID 83707, unpublished data). For this population, less aggressive options for recovery were precluded by the high, short-term risk of extinction. Although concerns remain regarding genetic changes associated with captive propagation and the possibility of catastrophic loss, the Ryman–Laikre effect is not an issue for the anadromous component of this population because the entire gene pool should be affected in a similar way.

For populations facing lower short-term risk, however, the strategy of taking the entire population in captivity is generally not a sound one. In such cases, there is one way, simple in concept, to simultaneously satisfy the divergent concerns regarding amplification of only part of the gene pool and placing a large portion of the population at risk: take fewer gametes (or progeny) from a larger segment of the population. For example, if our standard model had involved rearing in captivity several hundred progeny from each of 50 wild fish each year, instead of 1000 progeny from each of just 10, there would have been no differential enhancement of the gene pool during the captive phase. If the progeny (or gametes) could be obtained without undue risk to the natural population, the second concern would be satisfied as well. Because of the predominant effects of individual brood years under the crash scenario, even this strategy would not avoid increases in inbreeding, but it would reduce them.

Although simple in concept, this strategy will often be difficult to execute in practice. There are likely to be a number of technical and logistical difficulties in obtaining samples of progeny or gametes from a large segment of the population without placing it at undue risk. Nevertheless, a variety of approaches are possible, and one or more may be appropriate to any given captive broodstock program. Alternatives to taking adults from the wild to the hatchery include stripping some gametes from ripe fish and then allowing them to spawn naturally, collecting eyed eggs or swim-up fry from redds, and collecting juveniles as parr or smolts. Collecting juveniles has the potential to provide a random sample of the entire brood year and thus may represent the best general strategy for securing a broad genetic base for the captive broodstock program with little risk to the natural population. On the other hand, a sample of juveniles could also be derived from just a small portion of the adults that spawned, and it often will be difficult to determine whether a representative sample has been obtained. Furthermore, juveniles from the population of interest cannot always be separated reliably from juveniles from other populations in the area. Therefore, collection of juveniles can lead to population admixture, with possible consequences being reduction of diversity between populations and loss of local adaptation.

In many cases, a mixture of approaches may be the best strategy for initiating a captive broodstock program. Collection or stripping of adults or sampling redds would provide assurance that a known proportion of the entire brood year had been sampled. Supplementing these with a collection of juveniles should broaden the genetic base of the broodstock considerably, even if the juveniles were not a completely random sample of the entire brood year. A strategy similar to this is being used in the Dungeness River captive broodstock program (J. Shaklee, Washington Department of Fisheries, P.O. Box 43151, Olympia, WA 98504, personal communication).

One further strategy for broodstock collection deserves a brief discussion here. In this scenario, a decision would be made regarding the level of inbreeding (or the extent of a population bottleneck) that the managers of the program are willing to accept. As an example, assume that it had been determined that genetic risks from inbreeding would be acceptable as long as the population did not experience a genetic bottleneck equivalent to a single generation effective size of less than 100. For a salmon population with multiple age classes and average age at reproduction of 4 yr, this would be achieved if $N_b \geq 25$ per year. Given the results presented earlier, a conservative approach would be to assume that all of the contribution to effective size of the captive–wild system as a whole would have to come from the captive component. Thus, achieving the goal would require that $N_{bc} \geq 25$. To allow for the likelihood that the effective size would be less than the total population size, a target might be set to collect 50 fish per year. If $N_{bc}/N \geq 0.5$, this would result in $N_e \geq 100$ even if there was no contribution from the wild component.

This strategy seems reasonable because, according to Ryman and Laikre's (1991) formula (Equation 3), the effective size of the captive–wild system as a whole cannot be less than the effective size of either component. However, results depicted in Figures 5 and 6 show that this is not necessarily the case for Pacific salmon: under the crash scenario, the effects of age structure can cause the combined captive–wild population to experience an overall level of inbreeding higher than that predicted from the Ryman–Laikre formula and higher than that predicted from the captive component alone. Equivalent single-generation N_e values shown in Table 3 provide a convenient way of interpreting genetic changes associated with different captive broodstock scenarios, and these values can be factored into management strategies for broodstock collection.

Breeding and Rearing Strategies

In our simulations, sib-avoidance matings had little effect on the overall level of inbreeding in the postsupplementation population. Given this, one might ask what the benefits are to elaborate and resource-intensive mating schemes in the hatchery. There are two major reasons why such strategies may be desirable. First, although random mating in the hatchery did not affect the final level of inbreeding, it did result in a temporarily higher level than occurred under sib-avoidance mating. The significance of this increase cannot

be evaluated without making assumptions about the incidence of deleterious recessive alleles in the population. If such alleles are numerous, then the temporary increase in IBD may result in inbreeding depression and long-term reduction in population fitness. Strictly random mating might, therefore, entail some risk in a captive broodstock program for a depressed salmon population. The narrower the genetic base for the captive broodstock program, the greater this risk would be. For example, if L pairs of adults contribute equally to a group of progeny that mature in captivity, the expected proportion of full-sib matings among the progeny is $1/L$ if mating is random. Full-sib matings will occur infrequently if L is about 20 or more but can be a significant fraction if L is small.

Second, use of nested mating schemes (in which each individual is mated to two or more individuals of the opposite sex) has two advantages over stricly pairwise matings. A nested approach increases the number of different genotypic combinations in the progeny generation, thus providing a greater diversity of raw genetic material for natural selection to act upon. This may be beneficial to depressed populations that have experienced reductions in genetic diversity. In addition, a nested approach can help to minimize the adverse genetic consequences of individuals that produce inviable gametes. For example, if a particular male has inviable sperm, he would completely nullify the genetic contribution of one female under a pairwise mating scheme, whereas he would nullify only part of the gametes of two or more females under a nested mating scheme. The latter scenario would result in a lower variance among females in reproductive success and a higher effective population size. The nested matings, however, should be carried out without mixing gametes from individuals of the same sex (Gharrett and Shirley 1985; Withler 1988).

With these considerations in mind, we recommend the following general breeding strategies for captive broodstock programs. First, it is prudent to practice sib-avoidance matings to the extent possible. Often this can be accomplished with little additional requirements of time or resources by crossing individuals of different ages. In programs with a relatively large genetic base for the broodstock (N_{bc} about 15–20 or more per year), this line-crossing strategy should probably be sufficient to minimize short-term risks from inbreeding depression. However, line crossing will not be possible in the first and last years of a program, and unequal numbers of maturing adults from different brood years may reduce its usefulness in other years. Therefore, in programs based on a relatively small number of individuals per year, keeping track of individual family groups assumes a greater importance. This generally requires separate rearing, at least through a stage at which progeny can be marked, and may be very resource intensive.

Second, a nested or modified factorial mating design should be used with broodstock collected from the wild. Splitting gametes among two to five individuals of the opposite sex should be appropriate for most situations. In very small populations, a complete factorial cross should be considered (each individual mated to all individuals of the opposite sex). Another possible scenario is to divide the entire broodstock into a series of subgroups (with perhaps 4–10 individuals in each group) and conduct full factorial crosses within each subgroup. Factorial matings are less important for fish maturing in captivity because, if the program is successful, these should number in the hundreds or thousands. Nevertheless, mating each individual with two members of the opposite sex should be considered if feasible, if only for the possible fertility insurance provided by a backup male.

Cryopreservation of male gametes can also help to increase effective population size and reduce levels of inbreeding under certain circumstances. Cryopreservation may help to modify the edge effects described above and can serve as a safeguard in the early years of a program. The clearest advantage to cryopreservation is when it allows genetic material to be incorporated into the population from individuals that would not normally reproduce successfully. This might occur if only one sex is available for spawning at a given time. Under these circumstances, gametes from males could be cryopreserved for future use, and females could be fertilized with cryopreserved sperm. Because these instances are unpredictable, it would be useful to have a gene bank that included a supply of cryopreserved sperm from a number of males available for use with critically small populations. Ideally, males chosen for inclusion in such a gene bank should be among those that have not already made a significant genetic contribution to the population. Taking milt from wild males that have already spawned is one way this might be accomplished.

Cryopreservation could also be used routinely in a captive broodstock program to facilitate crosses between brood years. However, as discussed above, the long-term benefits of this strategy are uncertain. Furthermore, there is generally a loss in fertility associated with use of cryopreserved sperm (Cloud et al. 1990; Wheeler and Thorgaard 1991) that must be factored into any decision regarding its use. For example, low fertility rates have been found for cryopreserved sperm in captive broodstock programs for two endangered Pacific salmon species (Snake River sockeye salmon, K. Johnson, Idaho Department of Fish and Game, 1798 Trout Road, Eagle, ID, 83616, personal communication; Sacramento River winter-run chinook salmon, S. Foott, U.S. Fish and Wildlife Service, Box 2105, Anderson, CA 96007, personal communication).

Analytical and modeling results show that, for a successful captive broodstock program in which the population is rapidly expanding, it will not be possible to substantially augment effective population size by equalizing progeny number during captive rearing. This is because a bottleneck has already occurred in selection of only a small portion of the population for broodstock. On the other hand, a lower variance in progeny number does help to reduce inbreeding in stable or declining populations. Because the outcome of a captive broodstock program will be uncertain at the outset, program managers should consider using measures to minimize variance in progeny number that can be accomplished within reasonable logistic constraints.

Limitations of this Study

Although we have attempted to provide a comprehensive evaluation of the Ryman–Laikre effect on captive broodstock programs for Pacific salmon, it was not possible to consider every possible combination of parameter values. Similarly, there were some scenarios that we did not evaluate at all in this study. In this latter category are reduced success of hatchery fish spawning naturally, declining natural populations, and various alternative methods for spawning captively

reared fish (e.g., spawning them with fish captured from the wild or releasing them to spawn naturally). These alternatives could be considered in a susbequent study.

We also did not formally consider a variation of the crash scenario in which hatchery fish are distinguishable from wild fish and "excess" fish produced by the captive broodstock program are selectively harvested. In theory, this could result in a naturally spawning population of constant size with little increase in overall levels of inbreeding. However, captive broodstock rearing is unlikely to be a cost-effective means of producing fish directly for harvest. A scenario involving harvest of excess fish produced by a traditional, sea-ranching supplementation program is considered in a companion study (B.E. Riddell, Department of Fisheries and Oceans, Nanaimo, B.C., and R.S. Waples, National Marine Fisheries Service, Seattle, Wa, unpublished data).

Relatively little attention in this paper has focussed on the increase scenario. In one sense, this scenario is easily characterized because inbreeding levels in a population that remains large after supplementation will quickly become lower than those in the control. However, in contrast to the crash scenario, it is not possible to calculate a single value of ΔIBD for the increase scenario, because the magnitude of the departure from the control increases with time. Furthermore, in this study the increase scenario was compared with a control in which the population size remained small in spite of increases in the postsupplementation population. Static abundance in the control would be appropriate if there were multiple equilibria for population abundance and the natural population were "stuck" at a low abundance. Under this scenario, supplementation could be the catalyst to allow the population to reach a higher equilibrium. Possible mechanisms for this might be swamping of predators if their response is density dependent (Peterman 1987) or enriching nutrients in freshwater habit through decomposition of adult carcasses. However, if postsupplementation abundance were sustained because of permanent improvements to survival or carrying capacity of the habitat, the appropriate control population should also increase in abundance, although not so quickly as through captive broodstock enhancement. We have not quantitatively evaluated this scenario because it would involve considering an array of population growth curves.

Another factor not considered in this study is the significance of increases in IBD. That is, we have quantitatively evaluated levels of inbreeding but not the effects of inbreeding depression. Similarly, we have not attempted to evaluate the long-term significance to the population of the loss of genetic diversity. Although these are legitimate concerns, they are difficult to quantify without making numerous assumptions. Until these issues can be more formally addressed, captive broodstock programs should take actions that minimize the possibility for adverse genetic impacts of all types.

Genetic changes in the population that arise from differences between selective regimes in the captive and natural environments were also not considered in this study. These differences are not restricted to captive broodstock programs, as they also apply to any use of artificial propagation. Nevertheless, there are some distinctive features of captive broodstock programs that deserve a brief mention here. First, although captive broodstock programs involve rearing through an entire life cycle, this does not lead to an increased rate of genetic change in individual fish. Rather, the population as a whole may change genetically through differential survival of fish with different genotypes. Second, because selection cannot occur without differential reproductive success, a program that is successful in raising a high proportion of its captive progeny to maturity may largely avoid selection for a "hatchery" type fish during the captive generation. On the other hand, high survival in the hatchery would represent a significant relaxation of selection that occurs in the wild, so genetic combinations that are unfavorable for survival in the wild might be carried through the captive generation. It would not be surprising, therefore, to find that survival of first-generation progeny from a captive broodstock program was lower than that of either wild fish or fish released from a typical sea-ranching facility. Ideally, these unfavorable genetic combinations would quickly be weeded out and would not represent a long-term genetic load for the population. However, purging of unfavorable genetic combinations is unlikely to occur rapidly if the traits subject to selection are under polygenic control with genes having pleiotropic effects.

Finally, the values of CD and HP used in this study are consistent with a captive broodstock program that is successful in rapidly increasing (at least temporarily) the number of natural spawners. In practice, captive broodstock programs for Pacific salmon should still be regarded as experimental, with no guarantee of a boost in abundance for the natural population. At best, an unsuccessful captive broodstock program might have little permanent genetic impact on the natural population; at worst, it might substantially increase risk to the natural population through mining of broodstock, undesirable genetic changes, or both.

Because of the complex nature of captive broodstock programs for Pacific salmon and the impossibility of dealing with all important aspects in a single study, results presented here should not be used in isolation. Rather, they should be considered together with informed judgements on related issues to determine whether and how to implement captive broodstocks.

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Appendix

Let X be a random variable with binomial distribution (N, p), and let

$$Z = \frac{4X(N - X)}{N}$$

If X represents the total number of females from a total of N adults, then $Z = N_b$, the effective number of breeders (Wright 1931). Ignoring the trivial case in which $N < 2$ (and hence $Z = 0$), the expected value of Z is

$$E[Z] = \sum_{j=1}^{N-1} \frac{4j(N - j)}{N} \binom{N}{j} p^j(1 - p)^{N-j}$$

$$= 4\sum_{j=1}^{N-1} (N - 1)\binom{N - 2}{j - 1} p^j(1 - p)^{N-j}$$

By letting $k = j - 1$, one can write

$$E[Z] = 4(N - 1)p(1 - p)\sum_{k=0}^{N-2} \binom{N - 2}{k} p^k(1 - p)^{N-2-k}$$

Since the summation in the expression above is the binomial expansion of $(p + (1 - p))^{N-2} = 1$, one has $E[Z] = 4p(1 - p)(N - 1)$. If N is also a random variable, then the above expected value is conditioned on N. Thus, by taking the expectation again, we have $E[Z] = 4p(1 - p)(E[N] - 1)$. If, on average, sex ratio is equal ($p = 0.5$), and recalling that $Z = N_b$, then $E[N_b] = E[N] - 1$.

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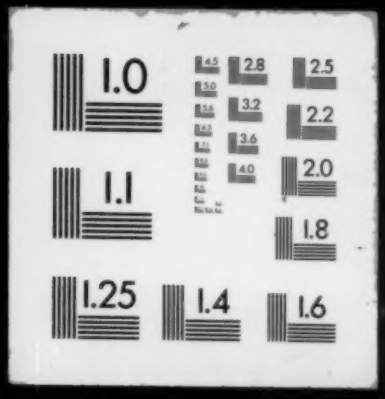
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